BRITISH COLUMBIA ACID MINE DRAINAGE TASK FORCE

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LITERATURE REVIEW FOR BIOLOGICAL MONITORING OF HEAVY METALS IN AQUATIC ENVIRONMENTS

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March 29, 1990

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Dear Mr. McKillop:

RE: LITERATURE REVIEW FOR BIOLOGICAL MONITORING OF HEAVY METALS IN AQUATIC ENVIRONMENTS

Please find enclosed, for your review and comment, seven (7) bound copies of our final report on the above project. We have included with the report a camera-ready copy and a copy of the report in WordPerfect 5.0 on an IBM compatible 5 1/4 inch diskette. Review of the first draft has resulted in the incorporation of an executive summary describing the project. We have included a flow chart and a glossary to aid the reader in understanding the overall organization and interaction between the various levels of testing available to study the impacts of acid mine drainage.

This study reviewed biological monitoring techniques for assessing the effects of heavy metals in freshwater environments, specifically for the impacts of acid mine drainage (AMD). The study provides a conceptual framework for organization of the available indicators, and information was compiled for bioassessment techniques ranging from biochemical through to ecosystem level. It was found that a variety of indicators are suitable for detecting the impacts of AMD, but few indicators have been linked to chronic, whole organism responses. No single approach to the problem of biological effects monitoring will completely meet the objectives of the B.C. Acid Mine Drainage Task Force. The most cost-effective approach to meeting the objective of assessing ecosystem health in waters affected by AMD would involve a multi-disciplinary, "top-down" approach. We recommend that follow-up field research be conducted to verify and develop this approach for wide application in monitoring the effects of acid mine drainage.

We have enjoyed working on this project and trust that it completes our present assignment to your satisfaction.

Yours truly,

E.V.S. CONSULTANTS LTD.

Kelly Munkittrick

K.R. Munkittrick, Ph.D
Project Manager
/me

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EXECUTIVE SUMMARY

The B.C. Acid Mine Drainage Task Force requested a complete review of the literature available on biological monitoring techniques related to heavy metals in aquatic environments. The review emphasizes a conceptual framework for organization of the various, available biochemical, population and community indicators. This framework allows for the future incorporation of new approaches. Few attempts were made to summarize the results found by various research teams; it was more important to determine that a change could be detected than to detail any controversy concerning the direction of that change.

This review provides the AMD Task Force with details of the wide range of available indicators. Many of these techniques have not been used specifically for examining the impacts of acid mine drainage, but there exist no *a priori* reasons why they should not be valuable as indicators of the impacts of AMD. Use of a battery or suite of biological assessment tests which assess responses at the levels of individual, population and community is strongly recommended; no one method or level of examination will provide all the required information. Biological indicators at all levels of organization are just that -indicators- and they require, for maximum utility, supporting data from analyses of tissue and environmental levels of contaminants, information about the history of exposure of the organisms to contaminated habitats, and some understanding of the vulnerability of the species to the particular contaminants.

Ecosystem health can be assessed by monitoring in two ways: a "bottom-up" or a "top-down" (reductionist) approach (Figure E1). Both approaches have advantages and disadvantages. Responses linked to detoxification processes (e.g., induction of MFOs in the case of selected organic contaminants, and metallothionein induction in the case of selected heavy metals) have a good conceptual basis for efficacy, since early changes in detoxification indices can generally be expected to be quite sensitive and precede the onset of more serious pathology at cellular and tissue levels of biological organization. Although cellular and biochemical measurements can be used as indicators of toxicant impact, and for helping to unravel the mechanism of effect, the significance of effects is best determined at higher levels of integration. It is often suggested that changes in simple biochemical/physiological responses may be useful for predicting the impacts of pollutants at population and community levels of biological organization. However, there are serious conceptual constraints to this approach. It seems likely that such simple responses can go no further than serving as early warning systems for delineating potential impact zones. Conclusions about cause and effect require demonstration of the source and pathway of impact, and a thorough knowledge of the biological behaviour and variability within a system. As a general rule, physicochemical criteria have probably been used with the least success in the long term management and conservation of natural ecosystems. Therefore, it is not advisable to regulate solely based on physiological changes in algae, invertebrates or fish.

The top-down approach initially monitors at the population or community level. Although there is a time lag associated with changes at this level of organization, the impacts have the advantages of integrating responses over a considerable period of time, and being relatively slow to reverse. Regardless of the approach taken, most improvements in monitoring and assessment programs have been related to the development of a comprehensive, multidisciplinary approach to problem-solving. No single approach to the problem of biological effects monitoring is fully satisfactory. Some methods are more useful than others, but the greatest insights are provided by multi-disciplinary efforts.

The best understanding of the effects of acid mine drainage will be reached through a progression of studies from the community level through to the level of biochemical responses within individuals. The disadvantages of many community and population methods, which include poor specificity and obscure dose-response relationships, can be addressed by follow-up testing involving the use of appropriate biochemical tests. A key factor becomes the identification of which biochemical tests are appropriate for use.

The most promising and proven biological monitoring techniques include the following:

- Benthic community responses have been well described for acid mine drainage, and should be the level at which preliminary measurements are conducted. Follow-up testing should involve an assessment of the consequences of a change in benthic communities for resident fish populations.

- Growth and reproductive parameters of fish populations are easily measured, and should be recorded during preliminary evaluations of impacts. These findings can be used to design a more complete, detailed monitoring program once the degree of impact has been established. Most morphological parameters are easily recorded, and should be monitored when other fish population data are being collected.

- Survival should only be measured once evidence designating a zone of impact is available. This evidence would be composed of clear changes in benthic communities, absence of species or decreased performance of fish populations within the impact zone. The testing should be designed to provide information on cause and effect relationships or the impact mechanism related to local conditions.

- Other changes at the individual, biochemical level are valuable components of many studies, and are extremely valuable for examining for the mechanism of disruption. The selection of biochemical indicators should be based upon the mechanism of toxic action.

  Measurements of gill histological changes are valuable, and have been linked to changes at the organismal level. Estimates of biochemical parameters of growth should be restricted to studies where an impact on growth has been demonstrated at the level of the whole organism. Haematological parameters should only be used if there is *a priori* evidence that impacts on blood constituents are expected. Some blood assays, such as the Na^+ loss bioassay, can be used to indicate potential problems during caged fish bioassays. Other blood parameters (K^+ and Cl^-) can be useful if combined with other indications of effects and relevance to whole organism responses. Bone metal levels can be used to indicate chronic exposure levels and gill, liver or kidney levels to illustrate recent, metabolic activity.

It is essential to relate changes in the fish and benthos to changes in the water quality parameters. Suitable parameters include temperature, turbidity, chlorophyll a, dissolved oxygen, nutrient concentrations, pH, sulphates, conductivity, hardness and metal concentrations.

The future objectives of the B.C. Acid Mine Drainage Task Force are to examine the impacts of acid mine drainage and to investigate whether water quality objectives are protective. These objectives can be met by implementing a tiered testing approach which utilizes the biomonitoring techniques recommended herein.

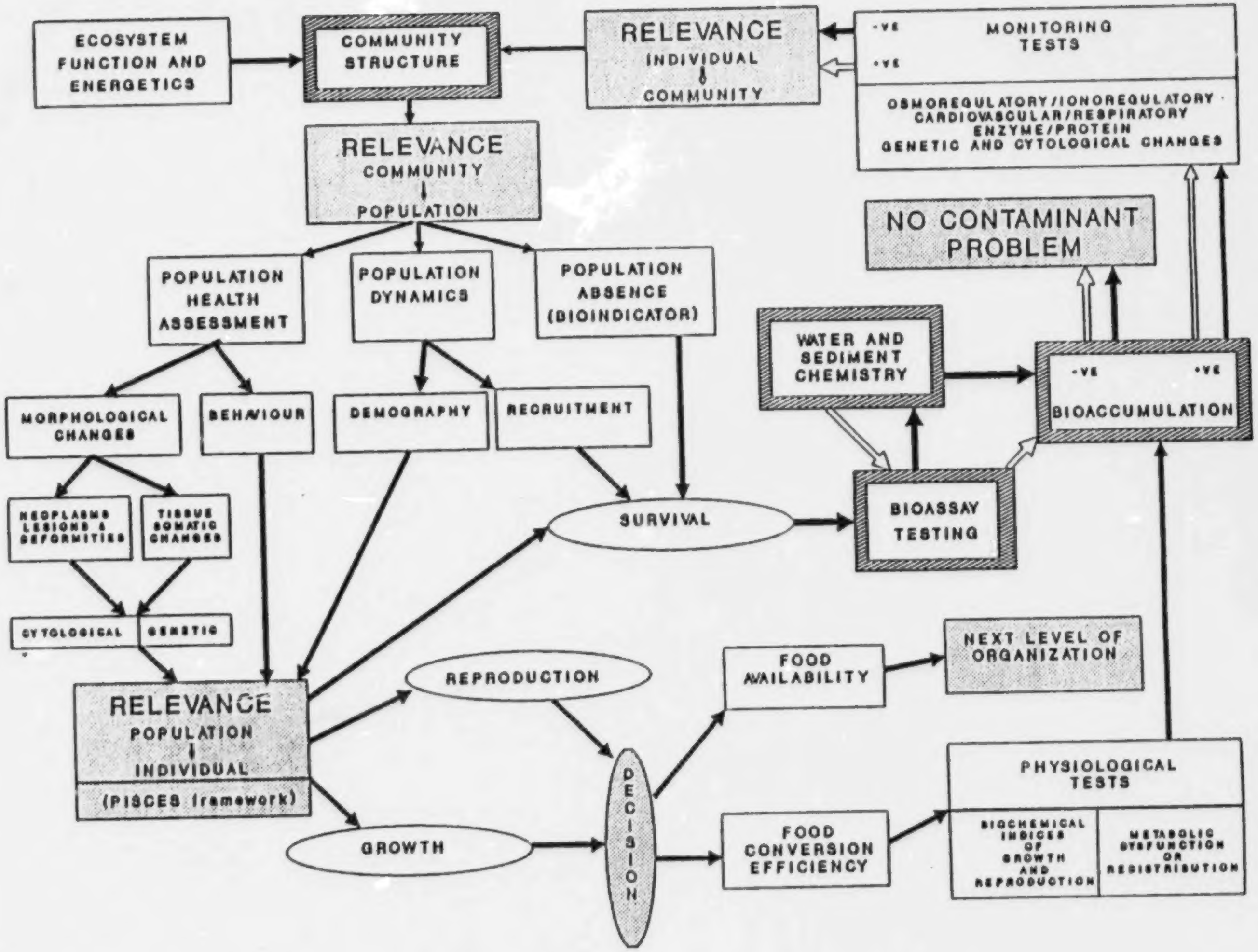
ECOSYSTEM FUNCTION AND ENERGETICS
COMMUNITY STRUCTURE
RELEVANCE INDIVIDUAL ↕ COMMUNITY
-VE
+VE
MONITORING TESTS
OSMOREGULATORY/IONOREGULATORY
CARDIOVASCULAR/RESPIRATORY
ENZYME/PROTEIN
GENETIC AND CYTOLOGICAL CHANGES
RELEVANCE COMMUNITY ↓ POPULATION
NO CONTAMINANT PROBLEM
POPULATION HEALTH ASSESSMENT
POPULATION DYNAMICS
POPULATION ABSENCE (BIOINDICATOR)
WATER AND SEDIMENT CHEMISTRY
-VE
+VE
BIOACCUMULATION
MORPHOLOGICAL CHANGES
BEHAVIOUR
DEMOGRAPHY
RECRUITMENT
NEOPLASMS LESIONS & DEFORMITIES
TISSUE SOMATIC CHANGES
SURVIVAL
BIOASSAY TESTING
CYTOLOGICAL
GENETIC
REPRODUCTION
FOOD AVAILABILITY
NEXT LEVEL OF ORGANIZATION
RELEVANCE POPULATION ↓ INDIVIDUAL
(PISCES framework)
DECISION
PHYSIOLOGICAL TESTS
GROWTH
FOOD CONVERSION EFFICIENCY
BIOCHEMICAL INDICES OF GROWTH AND REPRODUCTION
METABOLIC DYSFUNCTION OR REDISTRIBUTION

Figure E1. Impact assessment flowchart for detecting the impacts of acid mine drainage. The shaded boxes represent critical decision points and linkages in the assessment process. The cross-hatched boxes represent the monitoring tools currently in use for monitoring the impacts of acid mine drainage on aquatic systems (benthic community structure, bioassay testing, water and sediment chemistry and bioaccumulation studies). The clear arrows represent a top-down approach to monitoring, beginning at the biochemical level and progressing towards the community level. This approach can be used when problems are clearly defined, and links to the population and community level have been previously established. The closed arrows represent a top-down approach to be used when problems are poorly defined or relevance is not clear.

ACKNOWLEDGMENTS

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1.0 INTRODUCTION

Low pH and high levels of toxic heavy metals in acid mine drainage (AMD) are a serious threat to aquatic life, even at very dilute concentrations. Various techniques can be employed to detect the impact of dilute AMD or treated AMD effluent on the aquatic environment. Many of these, such as aquatic invertebrate surveys, detect an impact only after it has already happened (i.e., are retrogressive or reactive). Biochemical indicators, such as hepatic metallothionein, may help identify a potential impact and allow corrective action before impairment of aquatic systems occurs (i.e., may be proactive). Decisions regarding the selection of appropriate testing require compromise between the short time span and sensitivity of proactive techniques (e.g., biochemical indicators) and the ecological relevance and importance of reactive technology (e.g., community health assessment).

The B.C. Acid Mine Drainage Task Force has identified a need for a complete review and survey of the literature available on biological monitoring techniques related to heavy metals in aquatic environments, to evaluate their effectiveness and identify those which hold the greatest promise. Those with the greatest promise could then be adopted as standard techniques by the mining industry and government agencies. This review addresses this need.

1.1 PROBLEM STATEMENT

Acid Mine Drainage (AMD) conditions are fundamentally a problem of acid water produced by a combination of oxygenated water in contact with sulphidic ores. Iron-oxidizing bacteria (i.e., *Thiobacillus ferrooxidans, T. thiooxidans, Leptospirillum ferrooxidans, Sulfolobus brierleyii* (Silver, 1989) colonize in the presence of nutrients, and oxidize mineral sulphides to sulphate. In the presence of water, sulphuric acid is created. Metals in the ore are also released by this process, and the acidic waters maintain the metals at high concentrations in a dissolved and toxic form. Generation of acidic waters from the ore body requires contact with large surface areas relative to water volumes. This can be effected by passage of ground water through talus slopes containing ore, or from surface waters running over extensive ore outcroppings, overburden, or direct discharges of tailings. These possibilities should be considered in delineating the causal factors which result in "slugs" of degraded water quality which may lead to elevated metals concentrations, degraded benthic conditions, impaired fish reproduction or even fish kills (Vigers et al., 1983). Any mining operations which increase the reactive surface area of the ore body can further aggravate such situations. Such problems have been documented throughout the world in mining activities which involve sulphide ore bodies (i.e., coal deposits or pyritic ores).

While mines often cause problems during operation, contamination can continue to occur long after active mining has ceased. Mine tailings that have accumulated over several centuries of mining can still contribute to environmental degradation in local waters centuries later. Attempts to prevent impacts of AMD through various reclamation procedures have, to date, not been overly successful (Hammer, 1989).

1.2 OBJECTIVES

The primary objective of this work is to develop a list of the most promising and proven biomonitoring techniques which will:

- measure impacts of dilute AMD or treated AMD on the aquatic environment
- assist in confirming that permitted released of treated AMD, and available dilutions, are in fact protecting downstream aquatic resources
- assist in confirming that water quality objectives set for receiving waters are low enough to achieve the designated use to be protected.

2.0 APPROACH AND OVERVIEW

This review summarizes the available literature on aquatic biomonitoring techniques, with a focus on effects of low pH and elevations of heavy metal concentrations . The enormous toxicological data base for aquatic organisms generated over the last 40 years is very difficult to review in a formalized approach, even when the data are subdivided into a single group of organisms such as fish (Cairns et al., 1984). Assessment of the impacts of acid mine drainage is usually accomplished through chemical and toxicological (=bioassay) characterization augmented, in some cases, with *in situ* community measurements. Chronic toxicity data are not available for all components of acid mine drainage and, in the U.S., discharge limits are based on conservative not-to-exceed formulae given by the EPA, with an added safety factor to extrapolate from acute to chronic exposure effects. Evaluation is complicated by the uncertainties associated with simultaneous exposure to multiple contaminants and exposure from multiple pathways.

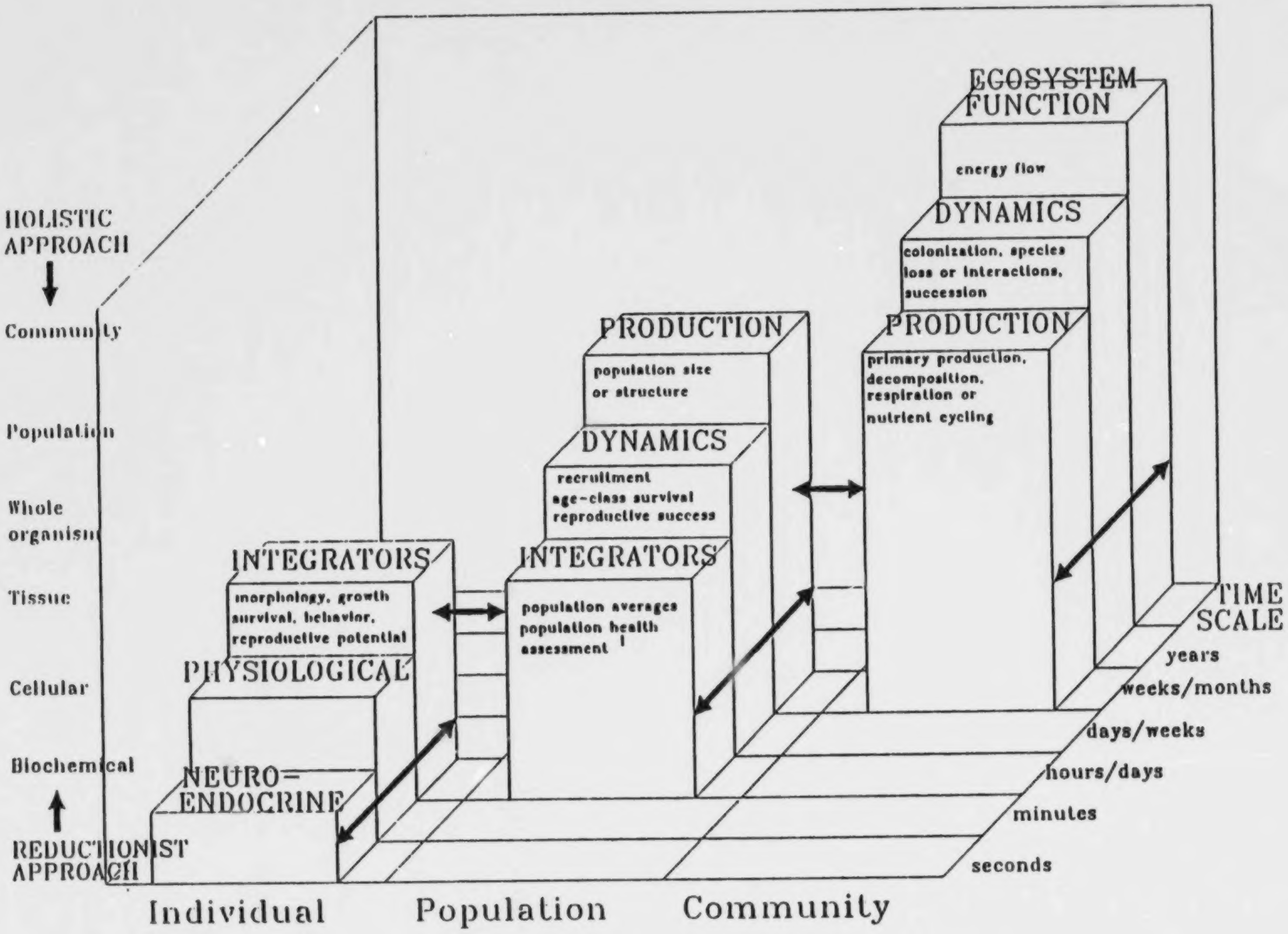
After conversations with members of the AMD Task Force, it became apparent that the most cost-effective approach for the review effort was to concentrate on summarizing the technology available for detecting the impacts of AMD on aquatic organisms. Few attempts were made to summarize the results found by various research teams; it was more important to determine that a change could be detected than to detail any controversy concerning the direction of that change. The review emphasized a conceptual framework for organization of the various, available biochemical, population and community indicators. This framework allows for the future incorporation of new approaches.

This review provides the AMD Task Force with details of the wide range of available indicators. Many of these techniques have not been used specifically for examining the impacts of acid mine drainage, but there exist no *a priori* reasons why they should not be valuable as indicators of the impacts of AMD. The approach taken for this review was to present a summary of the available techniques, with an overview of their advantages and disadvantages.

Bioassays are widely recognized as an assessment tool, but it is important to establish the ecological significance of sublethal effects (Heinz, 1989) and conduct field validation of bioassay methods. Biomarkers (indicators of exposure on a biochemical or cellular basis) include body burdens, indicators of DNA damage, adducts to molecular constituents (DNA or protein), enzyme induction or inhibition, histological changes, and biochemical indicators of reproductive or bioenergetic status. The ability of current methodologies to evaluate the ecological significance of toxicology and chemistry is the key to ecosystem health assessment.

Possible biological responses to AMD can be categorized into individual, population and community measurements. The stress response characteristically consists of primary, secondary and tertiary responses at the level of the individual, population and community (reviewed in Pickering, 1981; Cairns et al., 1984). At the individual level, primary and secondary changes are easily reversible, generalized responses which can lack long-lasting effects at the whole organism level. Tertiary, individual effects are not as reversible (Figure 1), consist of changes associated with reproduction, disease resistance and survival and seem to be the most meaningful in terms of population effects. Organismic changes work through individuals to result in changes in the overall characteristics of populations. Tertiary, individual changes would result in secondary changes at the population level and primary changes at the ecosystem level (Figure 1). Again, primary responses are the most reversible at the community level.

A conceptual framework, such as this, is required to evaluate the multitude of biomonitoring techniques, procedures and species, and identify those which are proven, or show the most promise for achieving the desired objectives. We have developed an impact assessment framework to categorize and organize the available, relevant literature (Figure 1). This framework gives an indication of the availability of data which could be of relevance to the concerns of the Task Force. The relevant, available data were reviewed in terms of this framework; relevant information was collated for presentation in narrative and tabular format, by major divisions. After categorization,



1 Population health assessment: use of biochemical or physiological responses to monitor or predict the overall health.

Figure 1. Impact Assessment Framework: Lowest impact levels, represented by the smallest boxes, respond to a low level of stress, have the fastest response time, the most reversibility, best early warning potential and worst diagnostic potential. The highest impact levels, represented by the largest boxes respond to the largest amount of stress, have the least reversibility, the longest response time, the best diagnostic potential and the lowest early warning potential. Arrows indicate direction of integration and prediction.

the data and principles are further developed in subsequent sections, and recommendations are made regarding the appropriate testing procedures for detecting acid mine drainage impacts on natural systems.

3.0 LITERATURE REVIEW

3.1 PHYSIOLOGICAL RESPONSES

The measurement of changes in the physiological responses of individual organisms forms an important component of any environmental monitoring program. Some biochemical responses can provide direct information on contaminant-induced changes, such as the induction of metallothionein or metallothionein-like proteins or changes in blood enzymes related to specific metal exposures. Other responses provide indirect information describing physiological status or non-specific responses to foreign chemicals, such as changes in adenylate energy charge. Indirect changes in haematology, such as decreases in haematocrit, leucocrit and mean corpuscular volume, and increases in haemoglobin concentration have also been used to characterize contaminant effects. For physiological responses to be useful measurements of biological effect in pollution studies, they should fulfil most of the following criteria (Widdows, 1985a):

- they should be sensitive to environmental stress and pollution and have a large scope for response throughout the range from optimal to lethal conditions
- they should reflect a quantitative or otherwise predictable relationship with the toxicant
- they should have a relatively short response time, on the order of hours to weeks, so that the toxicant impact may be detected in its incipient stages
- they should represent non-specific (general) responses to the sum of environmental stimuli, thus providing measurements of the overall impact of environmental change and complementing the more contaminant-specific responses at the cellular level
- they should be measurable with precision and with a high "signal to noise" ratio so that the effect of pollution may be detected above the "noise" of general variability

- they should have ecological relevance and be shown to be related to adverse or damaging effects on the population.

Perhaps the greatest potential weakness in the application of physiological techniques in biological effects monitoring concerns their variability (Bayne, 1985). Variability may be attributable to a range of sources such as seasonality, reproductive status and test conditions. Variability between individuals is not well studied.

This section of the report has been subdivided into the following sections for organizational purposes. Subsections deal with biochemical indicators of bioaccumulation, osmo- and ionoregulatory changes, cardiovascular and respiratory responses, enzyme and protein changes, biochemical indicators of growth and cytological changes.

3.1.1 Bioaccumulation

Bioaccumulation is a general term describing a process by which chemical substances accumulate in aquatic organisms, with the term bioconcentration referring to concentrations greater than those present in the external environment. Bioaccumulation integrates the overall process of chemical uptake and retention, and has been described by various authors through studies on uptake kinetics, metabolism and excretion/depuration, tissue distribution and compartmentalization, complexation, storage and bioconcentration. Bioconcentration is a consequence of exposure, but cannot be considered a true response, since few data exist that provide direct cause and effect evidence between tissue residue levels and chronic or sublethal effects. For most components of AMD, a given concentration has unknown consequences for the organism or the population, or on other species through community interactions.

This discussion of bioaccumulation is restricted to its suitability as an indicator of field exposure to contaminants. Therefore, little effort is spent to describe the uptake kinetics, depuration or metabolism of these metals associated with acid mine drainage. The discussion concentrates on the potential use of whole body, tissue and blood metal levels to indicate ambient metal levels.

3.1.1.1 Algae

Much information has been gathered concerning the use of algae and plants as monitors of heavy metal pollution related to their ability to bioaccumulate metals to levels several thousand times the concentrations measured in water (Bryan and Hummerstone, 1973; Foster, 1976; Baker and Brooks, 1989; Forsburg et al., 1988). Although work has primarily focused on macroalgae and terrestrial plants, the value of algae as indicators and integrators of ambient metal concentrations in aquatic environments has been established.

3.1.1.2 Whole Body

Not all components of acid mine drainage have the capacity to bioaccumulate and to bioconcentrate up the food chain (=biomagnify), and not all tissues have an equal capacity for uptake or storage. Many of the metals found to be elevated in acid mine drainage are essential micronutrients, and tissue residues will be dependent upon the organism, the chemical, the duration of the exposure and the tissues examined (i.e., Pastor et al., 1988; Miller et al., 1989a,b).

The strategies of heavy metal accumulation vary between species, and range from species which are capable of regulating a relatively constant internal body concentration over a wide range of external concentrations, to species which are capable of accumulating large quantities of metals and storing them internally in a detoxified form (Besser and Rabeni, 1987; Rainbow, 1989). Tissue levels can also be affected by trophic status (Wren and MacCrimmon, 1986) and chemical form (Kleinow and Brooks, 1986a). Krantzberg (1989) studied chironomid bioconcentration factors (tissue level divided by sediment concentration) for nine metals, and found that the most important properties determining concentration factors were ionization and oxidation potentials (i.e., measures of reactivity), ionization potential differential, and atomic radius (size). Concentration factors were higher for the essential elements Zn, Cu, and Ca than for Ni and the nonessential Pb, Cd, and Al. However, concentration factors were also low for the essential elements Fe and Mn, which may form insoluble and unavailable hydroxides.

The route for uptake and elimination of metals varies with both species and chemical. Uptake of contaminants may occur from solutions or from ingested particles (Watras et al., 1985; Kleinow and Brooks, 1986b). Long term (<13 d) exposure of *Daphnia* and algae to Ni indicated that direct uptake from solution rather than uptake from ingested algae was the primary accumulation vector. The increased concentration of Zn in the posterior intestine of white sucker (Miller et al., 1989a,b) is largely a reflection of adsorption of metals to diet constituents and the binding or adsorption of Cu and Zn to cuticular proteins in the exoskeletons of invertebrates (Milner, 1979; Dallinger and Kautzky, 1985; Krantzberg and Stokes, 1989).

3.1.1.3 Tissue Levels

There have been many attempts to use tissue metal levels to monitor the impacts of contaminated environments. Muscle metal burdens are used as an integrator of contaminant levels over long time periods. Limits on fish tissue levels of metal contaminants have been set to protect human health, and non-lethal methods have been described for sampling muscle (Skurdal et al., 1986). However, total metal levels in axial muscle may not be reflective of exposure concentrations. Variation between tissues in the tendency to accumulate metals has been widely documented and, despite elevated metal levels commonly found in metabolic tissues, levels in axial muscle may, in fact, be significantly higher in unexposed fish (cf., Miller et al., 1989a). Within the range of tolerance for nutritionally essential metals such as Mn, Fe, Zn and Cu, normal homeostatic mechanisms allow isolation of the muscular compartment from environmental elevations (Murphy et al., 1978; Giesy et al., 1980; Wilson, 1980; Miller et al., 1989a,b). Levels of metals in bone are relatively conservative, and elevations have been found to closely parallel the environmental concentrations (Bendell-Young et al., 1986; Miller et al., 1989b). Bendell-Young et al. (1986) caution that growth rates have to be taken into account for some metals.

For fish, not all tissues will show elevations after exposure to acid mine drainage. In a study of Cu and Zn contamination, white sucker (*Catostomus commersoni*) from contaminated sites showed significant increases in levels of both Cu and Zn in liver, kidney and gill tissue, but not in muscle and some other peripheral tissues (Miller et al., 1989a). Similar increases in levels of Cu and Zn in metabolic tissues have been reported for other species of fish taken from contaminated environments (Wilson, 1980; Roch and McCarter, 1984a). In juvenile rainbow trout, the highest concentration of Hg was found in the kidney or spleen, liver and kidney, while the largest relative increases were in the brain, muscle and kidney (Baatrup et al., 1986; Baatrup and Danscher, 1987). Cell injuries were closely related to the Hg tissue burden (Baatrup et al., 1986).

3.1.1.4 Blood Metal Levels

The distribution of metals, such as Zn and Cu, in erythrocytes and plasma has been described for a variety of species (Bettger et al., 1986), but the mechanism and metabolism are poorly understood for freshwater fishes. Bettger et al. (1986) found that exposed rainbow trout have high levels of Zn in plasma and erythrocyte membranes. Research is necessary to identify specific binding ligands for metals and to examine plasma metal homeostasis under a variety of environmental conditions.

Blood Pb is a valuable measure of Pb exposure since it directly reflects the amount of Pb that is biologically available (reviewed in Hodson et al., 1984; NRCC, 1985). As well, it is highly relevant to fish health because of its relationship to neurotoxicity. Field studies of the significance of neurotoxicity, as expressed in spinal curvature, are necessary to validate this and other indicators of Pb exposure, and are a high priority area of research. The problems include expense, complexity and sensitivity to sample contamination.

Blood Pb levels in fish increase proportionally with waterborne Pb levels, and are more convenient indicators of Pb exposure than whole body analysis because of lower detection limits and simplicity in tissue preparation. Reported blood Pb levels in fish range from less than 10 to 58,000 μg/L, but normal values for unexposed fish are less than 100 μg/L (NRCC, 1985). Chronic neurotoxicity in fish occurs at blood Pb levels less than 300 μg/L. The variability of blood Pb level tests is due to analytical error (SD of 11% of mean) and variation between fish. Much of the variability may be due to contamination of very small samples during handling. Results are usually reported as μg Pb per volume of whole blood; variability might be reduced by dividing the haematocrit to give μg of Pb per volume of red blood cells (reviewed in NRCC, 1985).

3.1.2 Osmoregulatory/Ionoregulatory changes

3.1.2.1 Whole body changes

Whole body ionic composition measurements in fish have been made to assess the influence of chronic acid exposure on ion regulation (Lacroix et al., 1985; Cunningham and Shuter, 1986). Ionic composition of juvenile Atlantic salmon (*Salmo salar*) was affected by pH; Ca, Na, Cl and Mg generally decreased, while K remained constant (Lacroix et al., 1985). Decreased ionic composition of juvenile Atlantic salmon exposed to acidic conditions corresponded to cessation of feeding behaviour, severe emaciation and growth retardation (Lacroix et al., 1985).

In young of the year smallmouth bass (*Micropterus dolomieui*), changes in body composition associated with starvation were similar in lab and field studies; the amount of water, ash, Ca, K and Na increased progressively in both laboratory and wild fish (Cunningham and Shuter, 1986). No pH effect was observed for levels greater than pH 5, but rates of decrease of each parameter were greater with lower pH values. The results do not suggest that chronic exposure to low pH conditions leads to significant increases in metabolic costs for young smallmouth bass. However, starvation may reduce the tolerance of the fish to low pH through progressive weakening of the osmoregulatory system (Cunningham and Shuter, 1986).

Whole body changes in ion levels are difficult to interpret without a considerable data base, and are difficult to relate to whole organism effects without carefully controlled experiments. A better estimate may be obtained through the examination of changes at the haematological level.

3.1.2.2 Haematological changes

Exposure to a variety of acidic conditions and metals have resulted in changes in ionic status of the blood or haemolymph. A number of studies are cited in Table 1. Although exposure to metals may alter both acid-base and ionoregulatory capabilities, there has been little success in linking these effects with whole organism changes. In studies with Zn exposure, ionic upsets did not appear to be sufficient to cause observed mortalities (Spry and Wood, 1985). The primary lethal mechanism might operate at the cellular level with the most likely effects either on oxygen delivery and/or utilization, or Ca homeostasis.

Measurements of blood parameters in fish collected from affected sites are related to reference (control) site values and variability is usually high. As well, there are differences in the effects of stress on blood parameters for different species and reproductive states (Lacroix et al., 1985). The impairment of ionic and osmotic regulation in fish are well documented responses to chronic acid exposure; haematocrit levels, plasma protein, electrolytes, and osmolality have been utilized as indicators of osmoregulatory ability, while ionic and/or blood fluid responses were used as indicators of ionoregulatory ability (Lacroix, 1985; Jones et al., 1987). Changes in glucose reflect the mobilization of energy reserves and may also act as an osmoregulatory mechanism (Jones et al., 1987).

Plasma Na, chloride, K and Ca are most commonly used as indicators of osmoregulatory function, and levels of Na, K and other ions usually decrease after exposure to acid (reviewed in Fromm, 1980; McKeown et al., 1985; Leino and McCormick, 1984; see also Table 2). Grippo and Dunson (1989) examined the rate of Na loss in fish exposed to acid mine drainage associated with coal mining waste. The fish were held *in situ* in enclosures or held under laboratory conditions with artificial stream water, and the authors concluded that the Na loss bioassay was a sensitive, reproducible, discriminating and cost-effective means of evaluating acid mine drainage. Although results with crayfish species also suggested that Na loss was a valuable parameter to monitor in some species of invertebrates, experiments with ephemeropteran and odonatan species did not show a relationship between acid tolerance and Na loss (reviewed in Berrill et al., 1987).

Table 1. Physiological function of clinical parameters in fish, and interpretation and physiological significance of alterations in these parameters (adapted from Larsson et al., 1985 and Wedemeyer et al., 1984).

Clinical Parameter	Physiological Function	Possible Physiological Significance	
		Low Values	High Values
Hematology			
Hematocrit	Oxygen carrying capacity in blood	Anemia; hemodilution due to gill damage or impaired osmoregulation	Polycythemia due to stress; hemoconcentration due to gill damage; dehydration
Hemoglobin	Oxygen carrying capacity in blood	Anemia; hemodilution due to gill damage or impaired osmoregulation; certain nutritional diseases	Polycythemia due to stress; hemoconcentration due to gill damage; dehydration
Red blood cell (RBC) counts	Oxygen carrying capacity in blood	Anemia; hemodilution due to gill damage or impaired osmoregulation	Polycythemia due to stress; hemoconcentration due to gill damage; dehydration
Leucocytes	Defense against foreign cells and matter	Leucopenia due to acute stress	Leucocytosis due to bacterial infection
MCHC (mean cellular hemoglobin concentration)	Status of red blood cells; reflects the supply of constituents for the hemoglobin synthesis	Hypochromic anemia; hemodilution	Polycythemia due to stress; hemoconcentration due to gill damage; dehydration
MCH (mean cellular hemoglobin content)	Status of red blood cells; reflects the supply of constituents for the hemoglobin synthesis	Microcytic, hypochromic anemia, often associated with iron deficiency	Macrocytosis
MCV (mean cellular volume)	Status of red blood cells; red blood cell size; reflects a normal or abnormal cell division during erythropoiesis	Shrunken red blood cells due to hypoxia, stress or impaired water balance; microcytic anemia	Swollen red blood cells due to impaired water balance (osmotic stress); macrocytic anemia
Clotting time	Prevent blood loss	Thrombocytopenia in acute stress	Sulfonamides or antibiotic disease treatments affecting the intestinal microflora
Erythrocytic ALA-D activity	Hemoglobin synthesis	Inhibited erythropoiesis; lead poisoning	Stimulated erythropoiesis
Lymphocytes	Immune defense; antibody production, cell mediated and humoral immunological responses	Lymphopenia due to acute stress or impaired production of new cells in blood-forming tissues	Lymphocytosis due to bacterial or virus infections or cell necrosis; acute bleedings

Table 1. Continued.

Clinical Parameter	Physiological Function	Possible Physiological Significance	
		Low Values	High Values
Neutrophilic granulocytes	Immune defense; first defense barrier against bacteria; fagocytosis; inflammatory responses	Neutropenia due to impaired production of new cells in blood-forming tissues	Neutrophilia due to bacterial infections, inflammations or cell and tissue damage; acute stress; severe exercise
Thrombocytes (spindle cells)	Blood clot formation	Thrombocytopenia due to chronic stress or certain infections	Thrombocytosis due to acute stress (e.g., asphyxia), acute blood loss, inflammatory conditions
Metabolism			
Liver somatic index (LSI)	Metabolism	Starvation or nutritional imbalance	High metabolic activity; induced mixed function oxidase system; sexual maturation (females)
Liver glycogen	Carbohydrate metabolism	Acute or chronic stress; inanition	Liver damage due to excessive vacuolization; dietary imbalance
Muscle glycogen	Carbohydrate metabolism	Acute or chronic stress; inanition	Dietary imbalance
Blood glucose	Carbohydrate metabolism	Hypoglycemia due to inanition, chronic stress or low liver glycogen content; impaired imbalance	Hyperglycemia due to acute stress or anoxia; acute liver damage; hormonal imbalance (e.g., insulin deficiency)
Blood lactate	Carbohydrate metabolism	No recognized significance	Acute or chronic stress; swimming fatigue
Total proteins in blood plasma	Transport of substances otherwise insoluble in blood; colloid osmotic pressure; blood clotting; immune defense (immunoglobulins)	Hemodilution; inanition; nutritional imbalance; kidney damage; certain liver damages; infectious diseases	Hemoconcentration; impaired water balance
RNA/DNA (muscle)	Protein synthesis	Impaired growth, chronic stress	Good growth
Cholesterol (plasma)	Precursor for steroid hormones, plasma membranes and other specialized molecules	Impaired lipid metabolism	Fish under chronic stress, dietary lipid imbalance

Table 1. Continued.

Clinical Parameter	Physiological Function	Possible Physiological Significance: Low Values	Possible Physiological Significance: High Values
Osmotic and Ionic regulation			
Muscle water content	Osmotic and ionic regulation	Disturbed osmotic balance	In freshwater; impaired water balance due to acute stress, kidney damage, increased water permeability of gills and skin
Osmolality (plasma)	Ion regulation	External parasite infestation, heavy metal exposure, hemodilution	Dehydration, salinity increases in excess of osmoregulatory capacity, diuresis, acidosis
Plasma sodium and chloride	Osmotic and ion regulation; maintenance of osmotic pressure in body fluids; acid-base balance	Hemodilution due to disturbed osmotic balance; impaired active ion uptake due to damaged gills; impaired ion retention in renal tubules	In freshwater; hemoconcentration due to disturbed water balance;
Plasma potassium	Ion regulation; muscle cell function	Impaired active ion uptake via the gills or the intestine; impaired renal ion retention	Hemolysis; tissue damage;
Plasma calcium	Ion regulation; membrane permeability; muscle and nerve cell function; skeletal bone metabolism; blood coagulation	In freshwater: kidney damage (impaired tubular reabsorption); impaired intestinal uptake	Tissue damage; sexual maturation (females)
Plasma magnesium	Ion regulation; metabolism; muscle cell function	In freshwater: disturbed kidney function	Renal failure (impaired excretion); sexual maturation; anoxia
Plasma inorganic phosphate	Skeletal bone metabolism; energy metabolism	Disturbed kidney function; nutritional imbalance	Acute stress; disturbed kidney function (phosphate retention)

Table 2. Response of blood parameters to acid or metal exposure.

Species	Parameter	Exposure	Response	Reference
Invertebrates				
Astacus astacus	Na^+	acid	decreased	Appleberg, 1985
	K^+		decreased	
	Cl^-		decreased	
	Ca^{+2}		fluctuated	
Orconectes propinquis	Na^+	acid	decreased	Wood & Rogano, 1986
	Cl^-		decreased	
	Ca^{+2}		increased	
Daphnia magna	hemoglobin	Pb, Zn, Cd	decreased/ no change	Berglind, 1986
Procambarus clarki	ammonia excretion	acid	increased	Mauro & Moore, 1987
Procambarus fallax				
Procambarus clarki *Oronectes rusticus*	oxygen uptake	acid	no change	Mauro & Moore, 1987
Daphnia magna	Na^+	Al + acid	decreased	Havas, 1985
	Cl^-		decreased	
	Ca^{+2}			
Fish				
Salvelinus alpinus	Ht	acid	increased	Jones et al., 1987
	protein		increased	
	glucose		increased	
Salvelinus fontinalis	glucose	acid	increased	Tam et al., 1987
Oncorhynchus kisutch	Na^+	acid	decreased	Parker & McKeown, 1987[b]
Notemigonus crysoleucas	Ht	Cd	no change	Benson et al., 1987
Notopterus notopterus	glucose			Verma et al., 1986
	lactate			
	cholesterol			
Salmo gairdneri *Catostomus commersoni*	H^+	acid	increased	Hobe, 1987
	Na^+		decreased	
	Cl^-		decreased	
	K^+		decreased	
	Ca^{+2}		decreased	
	Mg^{+2}		decreased	
	PO_4		decreased	
	SO_4^-		increased	

Table 2. Continued.

Species	Parameter	Exposure	Response	Reference
Salvelinus namaycush	Ca^{+2} K^+ Na^+ Mg^{+2} Cl^-	Al + acid	decreased decreased no change no change no change	Gunn & Noakes, 1987
Coregonus spp.	δ - ALAD glucose Na^+ K^+ Cl^-	Pb	decreased increased decreased unchanged no pattern	Haux et al., 1986
Platichthys flesus (freshwater-adapted)	Na^+ Cl^- K^+	Cu	decreased decreased no change	Stagg & Shuttleworth, 1982
Platichthys flesus (saltwater-adapted)	Na^+ Cl^- K^+	Cu	decreased decreased no change	Stagg & Shuttleworth, 1982
Catostomus commersoni	Ca^{+2} Mg^{+2} Na^+ Cl^-	acid	no change no change decreased decreased	Hobe et al., 1983
Catostomus commersoni	Na^+ Cl^-	acid	decreased decreased	Fraser & Harvey, 1984
Salmo gairdneri	Ht vitellogenin	acid	no change decreased	Parker & McKeown, 1987
Salmo gairdneri	Ht vitellogenin	calcium enriched H_2O	no change decreased	Parker & McKeown, 1987

Ht = hematocrit
No change = No significant change
δ-ALAD = δ-aminolevulinic acid dehydratase

Several detailed studies have examined the relationship between Na loss and fish responses (Lauren and McDonald, 1986; Fryer et al., 1988). Lauren and McDonald (1986) concluded that fish died when they had lost approximately 50-55% of their exchangeable Na pool and Lauren and McDonald (1987a) concluded that acclimation of trout to acid conditions depends on changes in Na transport and permeability. To address the problem of predicting interactions between pH and metals Reid and McDonald (1988) examined plasma Ca and Na ion influxes and effluxes in response to three metals with the objective of predicting how the various components of the toxic mixture might interact.

Niimi and Lowe-Jinde (1984) and Lowe-Jinde and Niimi (1986) have met with limited success in the use of changes in erythrocytes and leucocytes. Various blood cell abnormalities such as the presence of micronuclei, nuclear adducts and chromatin condensation in erythrocytes have been used to examine contaminant with variable success (Gill and Pant, 1986). Most of these methods have not been widely used or field verified.

Behaviourial responses (thigmotaxis, activity and attraction to food extract) have been poorly correlated with the blood parameters described above, which suggests that the ecological relevance of these measurements is limited. Jones et al. (1987) conclude that the levels of various blood constituents can be used to assess the degree of stress at the moment immediately prior to capture; the comparative ease of such studies makes them attractive, relative to expensive behaviourial testing.

3.1.3 Cardiovascular/Respiratory Systems

The use of cardiovascular/respiratory physiological responses in aquatic toxicology generally has one of three main objectives (reviewed by Klaverkamp, 1982). The first, defined as an "acute sublethal toxicity test", attempts to use these responses as sensitive and rapid tests of chemicals for estimating potential adverse environmental effects. The second, defined as a "true bioassay", uses these responses to indicate the presence and relative quantity of a chemical or group of chemicals in solution. The third, defined as "toxicology", determines critical sites and mechanisms of action of adverse effects produced by chemicals (Klaverkamp, 1982).

3.1.3.1 Respiratory Changes

3.1.3.1.1 Protozoans

Respiratory response have been measured in *Tetrahymena pyriformis* (Slabbert and Morgan, 1982) as a quick (e.g., 10 min) indicator of toxicity. Results recorded with the protozoan were within the same range reported for changes in fish opercular behaviour (Slabbert and Morgan, 1982) or bacterial growth inhibition assays (Slabbert and Grabow, 1986). Results were compared to fish opercular rhythms after exposure to metals, cyanide, parathion and pentachlorophenol (PCP), but the responses were not the same for ammonia or phenol. Several problems are evident with this technique, including its insensitivity to low concentrations of most toxicants.

3.1.3.1.2 Algae

Effects on respiration have been used as an indicator of pollutant stress in algae. Turbak et al. (1986) developed an algal bioassay which uses oxygen evolution as a measure of toxicity. Several types of algal bioassay systems involving the measurement of oxygen evolution have been developed; reductions in oxygen evolution have been documented for algae exposed to contaminants (Hollister and Walsh, 1973; Hendricks, 1978; Sheridan, 1978).

3.1.3.1.3 Fish

Investigations on the toxicity (i.e., mechanisms and sites of action) of chemicals has included hydrogen ions, Zn, and Cd, as well as many other classes of compounds. Studies on these compounds demonstrate that hypoxia caused by interference of a variety of gill respiratory mechanisms may be a common toxicological mechanism for many diverse classes of chemicals (reviewed by Klaverkamp, 1982). Examples of branchial mechanisms that could result in hypoxia include increased mucous secretion, depression of aerobic metabolic processes, increased coughing frequency, loss of oxygen sensory ability, skeletal muscle paralysis and vasoconstriction in secondary lamellae.

Fish cardiovascular physiological responses have limited use as true bioassays for measuring concentrations of chemicals. Toxicant levels required to induce significant changes can be as high as 70% of the lethal level for some compounds (Klaverkamp, 1982) and the impacts of additional stressors and the activity of cardiostimulants and depressors have not been widely studied. Environmental factors have not been investigated sufficiently to evaluate their effects. There have also been major differences in surgical procedures, vasculature cannulation, approaches to restraint and anaesthesia, expression of dosage, route of drug (chemical) and presentation of data (reviewed in Klaverkamp, 1982).

3.1.3.2 Changes in Blood Cells (Haematological)

The numbers and concentrations of circulating blood cells can be used for detecting and evaluating the significance of sublethal impacts on fish (reviewed in Sorenson and Bauer, 1983; Dick and Dixon, 1985). The numbers and ratios of circulating white blood cells has been shown to change after exposure to Cu (Dick and Dixon, 1985) and Se (Sorenson and Bauer, 1983), but not to Hg (Niimi and Lowe-Jinde, 1984). In addition, abnormalities in red blood cells can include increased (macrocystis) or decreased (microcystis) cell size, as well as increased or decreased pigmentation (hyper- and hypochromic), as well as achromia, anisocytosis, polychromatophylia and poikilocytosis (reviewed in Sorenson and Bauer, 1983).

3.1.4 Enzyme/Protein Changes

There are many ways in which structure and/or function can be disturbed by metals; these have been classified by Slater (1978; cited in Widdows, 1985a) into four main categories:

1. Depletion or stimulation of metabolites or coenzymes; this may be serious enough to produce a morphologically evident lesion in the cell (e.g., changes in energy charge leading to substantial alteration of the intracellular redox state).

2. Inhibition or stimulation of enzymes and other specific proteins; the result may be a disturbance of metabolic integration leading to damage of cellular function (e.g., induction of enzymes such as MFO and metallothionein).

3. Activation of a xenobiotic to a more toxic molecular species; many versions of this type of molecular conversion are mediated by MFO. The damage to DNA, proteins or membranes, which is produced by such an active metabolite will be a complex function of its chemical reactivity, its rate of production and the efficiency and availability of protective mechanisms.

4. Membrane disturbances; many toxic substances or their metabolites result in cell injury by reacting primarily with biological membranes (e.g., lysosome damage).

A wide variety of protein changes have been evaluated, and can generally be separated into two groups. Some protein changes are directly related to a specific chemical, such as the relationship between δ-aminolevulinic acid dehydratase (ALAD) activity and Pb exposure (see Section 3.1.4.1). Since Pb activity is directly responsible for inactivation of the ALAD enzyme, there is a very specific relationship between the two components. Other protein or enzyme changes cannot be directly related to any specific exposure incident, but represent non-specific or indirect measurements of some previous exposure to stressors (Section 3.1.4.2).

There is a strong negative correlation between the ALAD activity of rainbow trout and their blood levels; therefore, ALAD is considered a better measure of biologically available lead than analyses of waterborne lead (Hodson et al., 1984). Measurements of the inhibition of ALAD activity of fish are inexpensive, technically simple, rapid and specific for lead. However, monitoring of ALAD activity in field studies has only been partially successful because methods need to be species specific, migration of fish populations confounds comparisons to reference sites, and contamination by inorganic lead is rarely severe enough to provide a good test of the ALAD method (Hodson et al., 1984).

3.1.4.1 Direct Indicators

3.1.4.1.1 Enzyme Changes

δ-Aminolevulinic Acid Dehydratase Activity

δ-Aminolevulinic acid dehydratase (ALAD) activity condenses two molecules of aminolevulinic acid to form one molecule of porphobilinogen, a precursor of haeme rings used in haemoglobin synthesis. Due to its sulphydryl groups, ALAD activity is inhibited *in vitro* by many metals, but only Pb is inhibitory *in vivo*. Near lethal levels of Cu, Hg, Ag, Cd, Zn and PCBs did not inhibit erythrocytic or hepatic ALAD activity of fish (NRCC, 1985). Since Zn slightly activates fish ALAD activity *in vivo* (8-10%), ALAD inhibition may be biased by the simultaneous exposure of fish to Zn or by Zn contamination of glassware. ALAD may not be sensitive to alkylated forms of Pb (NRCC, 1985).

In the invertebrate, *Daphnia magna*, combinations of Pb/Cd and Pb/Zn were powerful inhibitors of ALAD; however, at the highest concentrations of Pb and Zn the activity of ALAD was enhanced (Berglind, 1986). All tertiary combinations of Pb, Cd and Zn inhibited ALAD activity (Berglind, 1986).

In fish, Pb-induced inhibition of ALAD occurs in liver, kidney, spleen and erythrocytes of fish, but the most convenient tissue to assay is blood (NRCC, 1985). Erythrocytic activity is inhibited at blood Pb levels below those causing anaemia, since haemoglobin formation is usually complete before red blood cells circulate. In laboratory studies, ALAD activity of fish decreases as total waterborne Pb levels increase (reviewed in NRCC, 1985). However, under field conditions, many biotic and environmental factors affect the bioavailability and uptake of Pb. ALAD inhibition can usually be correlated with blood Pb levels in fish.

Surveys of inshore benthic species in Lake Ontario revealed a poor relationship between Pb levels in fish and ALAD inhibition, due to increased variability of ALAD levels in wild fish and decreased variability in blood Pb levels (reviewed in NRCC, 1985). Inhibition does not usually result in anaemia; however, it does provide an indirect, "early warning" estimate of potential neurotoxicity in fish. Prevalence of neurotoxicity increases when ALAD is inhibited by 50% or more, and inhibition (less than one week) is evident well before neurotoxicity (greater than eight weeks). Schmitt et al. (1984) examined the activity of ALAD in a number of species of fish impacted by acid mine drainage. They expressed activity on the basis of haemoglobin content, DNA and protein, and found that ALAD activity was depressed by 62-67% downstream of the tailings site. Haux et al. (1986) found ALAD activity to be depressed 88% in fish from Pb-contaminated lakes, but found no obvious changes in overall performance of the fish. Schmitt et al. (1984) cautioned that, since the enzyme is usually present in the blood in excess of physiological requirements, inhibition should be used as an indicator of Pb exposure rather than as a toxic response.

ALAD activity varies with the assay techniques, temperature, substrate concentrations and pH, and reaction conditions must be established for each species. This method is most effective when levels of Pb in contaminated fish are well above those of control fish; a clear geographical separation is required between control and treatment sites to minimize the effects of migration (NRCC, 1985). The most accurate conclusions will be reached when blood Pb is measured simultaneously. More research is needed on ALAD activity of fish in areas of well defined Pb contamination. The influence of environmental Zn (as shown by blood Zn levels) should also be investigated since Zn is often a co-contaminant.

Other biochemical indicators have been shown to increase with Pb exposure, including the measurement of Zn protoporphyrin levels with the use of fluorometric techniques. However, the correlation of Zn protoporphyrin levels with blood Pb levels during chronic exposures of livestock to Pb has recently been questioned (Bratton et al., 1989).

Cholinesterase

Cholinesterase enzymes are classified as either specific acetylcholinesterase (AChE), butyrl cholinesterase enzymes (BChE) or nonspecific cholinesterase (ChE). AChE is found in skeletal muscle and in central and autonomic nervous systems of fish and it functions to hydrolyze and terminate the effects of the neurotransmitter, acetylcholine (NRCC, 1985). ChE is found in the heart, liver and serum, and may have a role in regulating ionic permeability of cellular membranes. Greater diversity in ChE occurs among invertebrates such as crustaceans and molluscs (Habig and Di Giulio, 1989). Further information on cholinesterase can be found in Duangsawasdi and Klaverkamp (1979), Klaverkamp and Hobden (1980) and Lockhart and Metner (1984). Variability in assay by spectrophotometric technique has been attributed to assay temperature, instrumentation, degree of solubilization of membrane-bound enzyme and the amount of lipids in a sample of serum (Fairbrother, 1989). Activity of the enzyme has been shown to change with growth, and can be affected by age, sex, endocrine function and reproductive state, body weight, and levels of MFO activity. In rainbow trout, the enzyme activity after exposure to organophosphate and carbamate pesticides, was affected by assay temperature and body size (Zinkl, 1989).

AChE and ChE are selectively inhibited by organophosphate and carbamate insecticides at low concentrations and are nonselectively inhibited by metals at higher concentrations (NRCC, 1985). In invertebrates, inhibition of these esterases produces a wide array of symptoms, including fasciculations of all skeletal muscle systems, spasms in the gastrointestinal tract, increased secretory activity of glands, increased urination, decreased heart rate, increased blood pressure, and many central nervous system effects such as tremors, convulsions and ataxia (reviewed in NRCC, 1985). Presumably these symptoms occur because of increases in acetylcholine levels at cholinergic neuroeffector sites.

Because brain and skeletal muscle represent critical target sites in mammals, the same organs are frequently sampled from fish to evaluate the adverse effects of insecticides. However, there is generally a poor relationship between brain and skeletal muscle cholinesterase and survival/mortality data (NRCC, 1985). In field studies, Delorme et al. (1989) concluded that walleye exposed to sublethal doses of malathion showed a very good correlation with enzyme inhibition, but not with effects on survival, growth or feeding. Use of these enzymes for metal exposure has been poorly developed. Some field studies have indicated that the range of values in wild populations requires large sample sizes to detect differences (Lockhart and Metner, 1984). Compared to laboratory studies where standard deviations were on the order of 10% of the mean values, field studies have a greater spread of values, and therefore larger sample sizes are needed to detect the same degree of effect demonstrated in laboratory tests. Due to the increase in variability, Lockhart and Metner (1984) calculated that the field sample would have to be nine times larger than the laboratory sample to detect a significant difference; in order for biochemical methods to be practical tools in environmental assessment, they must be applicable to field populations.

Serum cholinesterase analyses are generally conducted as exposure indicators only. The time frame for response is generally within minutes of exposure, especially for the direct inhibitors which do not require biological transformation to react with cholinesterase. Organs and tissues can be frozen for relatively long periods of time without significant loss of cholinesterase activity. As with most enzymes, this activity is dependent upon temperature, pH, enzyme and substrate concentrations, buffer composition and other physical/chemical experimental variables. There are notable species differences and ranges of activity between tissues and organs within a species. Environmental factors have not been investigated sufficiently to evaluate their effects.

3.1.4.1.2 Protein Changes

Metallothionein

Metallothionein is included in this section although it is not a true direct indicator; high metallothionein levels cannot be related directly to any one metal being elevated, but are commonly used to indicate elevated metals. There has been some indication that levels can be influenced by other stressors (NRCC, 1985), and that, at least in mammals, metallothionein may function as a stress protein (cf. section 3.1.4.2.1). The literature on metallothioneins has expanded greatly since 1980; the following is a general discussion based on data found in Klaverkamp et al. (1984), Roch and McCarter (1984a,b,c), NRCC (1985), Olsson and Haux (1985, 1986), Hamilton and Mehrle (1986), Kay et al. (1986), Kito et al. (1986), and Klaverkamp and Duncan (1987).

Metallothionein is a low molecular weight metalloprotein or group of metalloproteins containing a high percentage of cysteine residues. The protein is ubiquitously distributed throughout plant and animal kingdoms, and is found in most physiological systems of invertebrates and vertebrates. Elevated rates of metallothionein synthesis and production are associated with exposure to heavy metals of Groups IB and IIB in the Periodic Chart. Metallothionein is thought to function primarily in the regulation of intracellular metabolism of metals, especially Zn and Cu. Proposed biological functions for this protein include the storage, transport and detoxification of essential metals, as well as some nonessential metals, including Cd and Hg. It is also possible that metallothionein may function as a regulator of hepatic metal concentrations during the reproductive cycle (Olsson et al., 1986).

Sublethal concentrations of some heavy metals produce elevated metallothionein concentrations within a week or less, in a dose dependent manner. There is a direct relationship between tissue concentration and response and this protein is thought to act by sequestering metals and reducing their availability to critical biochemical target sites. While not fully accepted as a metal detoxifying mechanism, there is a strong correlation between acclimation to metal toxicity and the amount of metallothionein.

Research has shown that metallothionein production takes place in an number of tissues, including gill, liver and kidney (Kay et al., 1986), that induction shows different sensitivity to different metals (Duncan and Klaverkamp, 1983; Klaverkamp and Duncan, 1987), several forms or isoforms of the protein may be induced (Kay et al., 1986; Kito et al., 1986; Price-Haughley et al., 1986; Benson and Birge, 1986), and additional proteins may be involved in metal metabolism (Harrison and Lam, 1986; Price-Haughley et al., 1986)

The "spill over hypothesis" was originally proposed to describe increased toxicity after the saturation of the binding capacity of metallothionein. Theoretically, the excess metal, left over after saturation of the metallothionein, was capable of interacting with enzyme substrates to disturb metabolism. This hypothesis is no longer widely accepted, and there is also some controversy over the use of metallothionein concentration as an indication of effects. It is now believed that the rate of metallothionein production, not the concentration, determines the degree of protection against metal toxicity (McCarter and Roch, 1984a). Elevated metallothionein concentrations are found in animals that are not acclimated to metal toxicity and metal concentrations in the high molecular weight fraction can increase in parallel with increased metal concentration in metallothionein.

While some evidence exists that there are important biological costs associated with increased production of metallothionein, such as reduced growth, more research is required to resolve this issue (cf. Klaverkamp et al., 1984). Roch et al. (1986) found good agreement between metallothionein levels and estimates of no-effect levels for metals. The relevance of metallothionein in producing metal-resistant populations of fish and other aquatic organisms needs to be established. Recent field investigations, however, do indicate that metallothionein holds considerable promise as an indicator of metal exposure and acclimation to metal toxicity.

3.1.4.2 Indirect Indicators

Physiological indicators of tissue activity levels and the occurrence of organ-specific enzymes in blood plasma or serum have been used successfully to illustrate the impact of toxicants on aquatic organisms. Indirect indicators can involve:

1) monitoring the products of enzyme activity (such as serum corticosteroid levels, stress proteins or brain neuroamine levels), or

2) examining the activity of enzymes. Studies involving determinations of enzyme activity levels can be divided into:

    a) enzymes measured in their natural *in vivo* active state (e.g., hepatic mixed function oxidase or brain monoamine oxidase activity), and
    b) serum enzyme levels, where increased serum levels of active enzymes are used to infer some damage to the parental tissues, which has led to the increased levels in circulation. Examples include plasma aspartate aminotransaminase (ALT), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alanine aminotransaminase (AST), lactate and malate dehydrogenases, cytochrome oxidase and alpha-amylase (Verma et al., 1986; Benson et al., 1987; Mizrahi and Achituv, 1989).

The more common applications and limitations of indirect indicators are described in the following pages.

3.1.4.2.1 Protein Levels

Corticosteroids

The imposition of a stressor (regardless of the type) will result in a primary stress response by that organism (see Pickering, 1981). This response involves, in fish, both the increased production and release of corticosteroids and catecholamines. With respect to corticosteroids, the degree of impact of the stressor can be quantified by monitoring increases in plasma cortisol concentration or interregnal nuclear diameter. These parameters have been used to assess the degree of fish response to a number of stressors (toxicants, aquacultural conditions, disease) and could theoretically be used to assess the overall condition of a population (Donaldson et al., 1984). Since any perceived stressor will alter these parameters, measures can integrate the overall impact resulting from several simultaneously applied stressors (Pickering, 1981).

In general, plasma cortisol concentration, as measured by radioimmunoassay, has been used to assess acute stress. Jones et al. (1987) and Tam et al. (1987) used blood cortisol levels as a measure of activity within the hypothalamic-pituitary-interregnal axis in response to stress. Jones et al. (1987) correlated changes in cortisol levels to behaviourial responses, but found that cortisol levels could not be directly related to treatment conditions alone. Closely related physiological changes associated with cortisol release are sometimes substituted because of the longer response period. Such related changes include serum glucose levels (which increase with cortisol release) and mean interregnal nuclear diameter (which increases under chronic cortisol production by the interregnal cells) (Brown et al., 1984; Donaldson et al., 1984). In salmonids, both parameters have been shown to respond to crowding in hatcheries, to toxicant exposure (copper, Zn, Cr, Hg, pulp mill effluent, 2,4-D, endrin) and to disease state (*Saprolegnia* infestation, bacterial kidney disease, bacterial gill disease) (NRCC, 1985).

Any factor which exerts stress on an organism, including handling, shallow water, anaesthesia, blood sampling, temperature shock, electric shock, hypoxia, confinement and salt water to freshwater transfer (or the reverse), will rapidly alter plasma cortisol levels (NRCC, 1985). Interregnal nuclear diameter is not as susceptible to these short-term stressors, since it takes longer to respond. Long-term biological and environmental stress modifiers (age, reproductive status, photoperiod) will alter both parameters. Due to high variability and the changes resulting from routine application in situations where the influence of potential biological and environmental factors is well defined, there is a problem with interpretation. Since the first stage of physiological homeostasis is being monitored, it is difficult to determine whether a response is acceptable (within the normal adaptive range of the organisms) or detrimental (falls outside the normal range). There would appear to be little ability to correlate changes in these primary stress indicators to significant impacts at the population level.

Stress proteins

Stress causes protein damage and breakdown, increasing the metabolic energy requirements of organisms. The stress protein response has been described for a wide variety of organisms; there is an increased production of high molecular weight "stress proteins" in response to a stressor. The response has been found for a number of stressors, including heat, heavy metals, oxidative stress and hepatic tumours (Sanders, 1989), as well as biological stressors. Stress tests are difficult to use under field conditions and require that the physiological conditions of the test organisms be controlled.

Brain neuroamine levels

Amines have received considerable attention recently with respect to their potential as indicators of the impacts of organic toxicants on fish. There has been some concern over the utility of whole brain amine levels as indicators of physiological processes. In teleosts, dopamine and/or serotonin have been associated with a number of other physiological processes, including the regulation of prolactin cell activity, melanocyte-stimulating hormone release and gonadotropin secretion, while noradrenaline levels have been implicated in thermoregulatory behaviour (reviewed in Munkittrick et al., 1989). The activity and specificity of the amines varies with season, timing and species.

Hyperactivity of fish has been associated with changes in the whole brain levels of noradrenaline and dopamine, or changes in noradrenaline, dopamine and serotonin (reviewed in Holdway and Dixon, 1986; Munkittrick et al., 1989). Changes in whole brain dopamine and serotonin levels have also been associated with uncontrolled twitching and motor dysfunction and decreased serotonin levels has been associated with abnormal swim bladder function (Holdway et al., 1986; Holdway and Dixon 1986).

Since the hormonal and behaviourial regulatory function of amines involve only very small nuclear or paracellular areas within the brain, whole brain indications of amine levels may reveal little about specific abnormalities due to a lack of basic information regarding storage sites and neurotransmitter function in fish (Munkittrick et al., 1989). It is not practical during field collections to sample microscopic areas of fish brains, although such sampling would be required in laboratory studies designed to elucidate amine function. In spite of this limitation, gross indications of whole brain amine status have been used successfully to demonstrate detrimental impacts of exposure of fish to PCBs, PAH, DDT, parathion and methoxychlor (reviewed in Sloley et al., 1986a,b; Munkittrick et al., 1989). It is presently unclear whether a cause and effect relationship exists between changes in amine levels and changes in the physical performance of fish. All of the above examples describe the impacts of organic chemicals which may play a role in disruption of the metabolism of the amines, as well as the physical performance of the fish.

The use of brain neuroamine levels for examining the impacts of acid mine waste on white sucker populations was inconclusive; the results were interpreted as confirmation of the absence of adverse physiological impacts (Munkittrick et al., 1989). The lack of a significant difference between sites was consistent with previous sampling collections which showed few major differences between sites in any physiological parameters measured. Significant seasonal and circadian changes indicate that collections from wild fish must be carefully standardized. Regardless of whether a cause-effect relationship can be demonstrated, whole brain amine levels may have some value as a general indicator of contaminant impact (NRCC, 1985). Information is required on normal seasonal changes in amine levels before information from field studies can be properly interpreted. Seasonal changes have been documented in feral salmonids (Sloley et al., 1986b) and reproductive state was shown to alter amine profiles in laboratory flagfish (*Jordanella floridae*) (Holdway et al., 1988).

3.1.4.2.2 *In vivo* Enzyme Activity

Hepatic Mixed Function Oxidase (MFO) Activity

The mixed function oxidase (MFO) enzymes belong to a very large group of membrane-bound enzymes which act to metabolize highly lipophilic, poorly-excreted, multi-ringed compounds to more hydrophillic byproducts that are more easily excreted. Enzyme activity can be divided into two groups, phase I enzymes which act to modify the structure of the parent compound (i.e., hydroxylation), and phase II compounds which conjugate various compounds onto the parent compound. Synonyms are cytochrome P-450 and P-448 enzymes, referring to the absorbance of the cytochromes responsible for the enzymatic activity. Both reactions result in an increase in hydrophillicity and excretion rate of the compound. A more detailed description of the enzymes may be found in other sources (NRCC, 1985; Lech et al., 1982). Techniques have been developed for determining MFO activity in amphibians and invertebrates (reviewed in Payne, 1984).

Potential inducers of the system have not been clearly identified, although the level of catalytic activity has been stimulated to near maximal levels by a wide variety of compounds (reviewed in NRCC, 1985). There are a wide variety of enzymes and substrates which are used to characterize MFO activity, including EROD, PROD, PNOD, ECOD, PPO, AHH, EMD, BeND, APND, and conjugation enzymes for various compounds such as glucuronide, sulphate, glutathione and taurine. Induction is rapid in fish, reaching a peak within 49 to 96 h of exposure to a contaminant. When the exposure is removed, activity may decline rapidly (within 7 to 10 d; Luxon and Cairns, in NRCC, 1985) or slowly (less than one year; Payne and Penrose, 1975). Induction is dose dependent, but dose response curves for most compounds are not available, and there may be a plateau of induction due to saturation of receptor sites and enzyme synthesis.

Payne (1984) cites several field studies where the exposure of fish to environmental contaminants (petroleum hydrocarbons, sewage) resulted in increased liver-enzyme activities. MFO activity may be used to identify or delineate broad geographical areas of mixed organic pollution (Payne, 1984). Measurements of MFO activity in wild fish have been related to exposure to oil spills (Payne and Penrose, 1975; Stegeman 1978), petroleum refinery effluent (Ridlington et al., 1982), polluted rivers in Europe (Kurelic et al., 1979) and municipal wastewater (Förlin and Hansson, 1982). Its utility for examining metal-based pollution has been unproven, although inhibition of MFO activity has been associated with metal exposure, or related hepatic necrosis (Ahokas et al., 1976). Despite the elevation of MFO activity in fish populations exhibiting a high prevalence of neoplasms and reproductive impairment, links have not been clearly established.

MFO activity varies with a variety of biotic modifying factors including: gender, age, liver size, fish species and strain, migration, ration level, dietary contaminants and dietary protein level (reviewed in James and Bend, 1980; Spies et al., 1982; McKee et al., 1983; NRCC, 1985; Luxon et al., 1987). These sources of variability can be reduced by measurement of factors causing variability, by sampling design and by statistical manipulations, providing that a sufficient number of measurements are made. Activity levels are also affected by contamination with blood or bile, and MFO assays should be conducted soon after the fish are captured, since holding time in clean water decreases activity, as does prolonged storage (NRCC, 1985). There is currently no method available for estimating the accuracy or precision of results and, given the high variability, it is difficult to detect differences in activity without very large sample sizes. While fish sampling methods do not appear to affect MFO activity, use of MS-222 immediately before liver sampling will reduce BaP hydroxylase activity (Fabacher, 1982).

Brain Monoamine Oxidase

Monoamine oxidase is involved in the metabolism of biogenic amines, transmitters which are used to coordinate many physiological processes. Altered metabolism results in changes in the levels of amines and indoleamines in neural tissue, which can be related to a number of changes in fish (reviewed in Munkittrick et al., 1989). Heavy metal ions are thought to inhibit the enzyme's activity by binding with sulphydryl groups (Unni and Caspers, 1985).

Neurotoxic effects of mercurials are well established; reduction of brain MAO induced by mercurials is indirect evidence of impairment of the aminergic system, and Hg is known to alter brain monoamine oxidase (MAO) synthesis in mammals (Ram and Sathyanesan, 1985). Little work has been done in fishes. For the teleost, *Channa punctatus*, exposure to mercuric chloride and Emisan (an organic Hg fungicide) over a 80 d exposure period had significant reductions in MAO (Ram and Sathyanesan, 1985). In addition, reproductive development was delayed in fish in the exposed group, which may be at least in part due to the impaired monoaminergic system responsible for modulating the hypothalamo-hypophysial-gonadal axis (Ram and Sathyanesan, 1985).

ATPase Activity

ATPase activity has been monitored in gill, brain and mitochondria to monitor impacts of metals. ATPases are widely distributed enzymes which are involved in the energy-requiring active transport of electrolytes across membranes (Passino, 1984).

Na^+/K^+-ATPase is the prime mediator of ion transport across cellular membranes and plays a central role in whole body ion regulation in marine and estuarine animals (Towle, 1981; cited in Torreblanca et al., 1989). A possible mechanism of acid stress is inhibition of gill Na^+/K^+-ATPase activities (Powell and McKeown, 1986). The response of gill Na^+/K^+-ATPase activity to heavy metals or acid exposure can vary with developmental stages (Beckman and Zaugg, 1988), the length of exposure and with pH (Powell and McKeown, 1986). Although the data base is relatively small, Na^+/K^+-ATPase is a potentially useful indicator of pollution stress in aquatic animals (Torreblanca et al., 1989). However, ATPase activities are not a valuable indicator of sublethal metal toxicity in the absence of other supporting indicators. Caution is again advised; although Laurén and McDonald (1987b) found that ATPase activity in the gill decreased by 33% within 24 h of exposure, this was compensated for by an increase in microsomal protein such that activity per mg of gill tissue returned to normal within 14 d.

A great deal of attention has been paid to gill ATPase enzymes, because of their importance in plasma ionoregulation. Additional studies have examined the activity of ATPase enzymes in other tissues, including fish brains, where the ATPase enzymes act to regulate intra-neuronal ion concentrations. Such regulation is required for proper transmission of nerve signals. Hg and Cd caused a significant reduction in Ca ATPase in catfish brains, disrupting synaptic transmission (Reddy et al., 1988).

Mitochondrial ATPase activity of several fish tissues was inhibited by DDT exposure (Passino, 1984). There is no reason to suspect that the enzyme would not also serve to indicate the impact of metals on metabolism.

Other *In situ* Enzymes

Hepatic allantoinase activity was found to be lower in larger lake trout (*Salvelinus namayacush*) than in younger lake trout collected from Lake Michigan (Passino, 1984). The author hypothesized that decreased activity was related to higher contaminant burdens in older fish, although the experiment was hampered by many confounding factors. Versteeg and Giesy (1986) used serum lactate dehydrogenase activity to examine Cd impacts on fish, but failed to detect a significant impact.

3.1.4.2.3 Misplaced Enzymes as Indicators of Toxicant Impact

Leucine Aminonapthylamidase Activity

Leucine aminonapthylamidase (l-aminonapthylamidase; LAN) is a proteolytic enzyme normally restricted to lysosomes (Dixon et al., 1985). Rupture or increased permeability of the lysosomal membrane results in release of the enzymes to the cytosol where they function in autolysis. Lysosomal membrane fragility is suitable for indicating acute impact of organic contaminants (Pellerin-Massicotte et al., 1989). Many substances (heavy metals, free radicals, peroxides, ionizing radiation, polycyclic aromatic hydrocarbons) are known to alter the lysosomal membrane structure resulting in destabilization and subsequent release of the lysosomal enzymes (NRCC, 1985). Lysosomal stability and the latency of the lysosomal enzymes can therefore be used to assess the extent of environmental contaminant impact, particularly in situations with multiple stresses (NRCC, 1985).

NRCC (1985) felt that the best indicator for determining lysosomal stability was the concentration of LAN in the blood of fish, since increased serum levels would be an indicator of cell death. Plasma sampling must be processed rapidly, since LAN in blood plasma is altered rapidly by time and temperature (Dixon et al., 1985). Plasma LAN levels have been shown to respond to dietary, intraperitoneal and waterborne stress in laboratory studies. Plasma LAN levels in fish are unaffected by handling such as electroshocking, netting and blood sampling, although levels can increase with age (Bouck, 1984). Its utility is further restricted by the high variability and instability, which necessitate the rapid sampling of large numbers of fish. Field trials with fish have yet to be conducted.

Other lysosomal enzymes which have been used include serum acid phosphatase and *N*-acetyl-β-D-glucosaminidase (Versteeg and Giesy, 1986).

Sorbitol Dehydrogenase

Sorbitol dehydrogenase (SSDH) catalyzes the reversible interconversion of fructose and the polyhydric alcohol sorbitol, an oxidation-reduction reaction which occurs predominantly in the liver (Wolf et al., 1973). Dixon et al. (1987) reported that hepatic SDH activity was an order of magnitude higher than that found in other fish tissues. Elevated levels of serum SDH (SSDH) have not been reported in organ disease other than liver insult, and they are considered in mammals to be liver-specific (NRCC, 1985; Dixon et al., 1987). None of the potential modifying factors evaluated to date (including starvation, gender, fish size and handling stress) have resulted in a significant change in SSDH activity. Data have been obtained for a wide number of organic chemicals using rainbow trout, and SSDH levels have been shown to increase after exposure to waterborne Cu (Dixon et al., 1987). SSDH responded quicker, and at lower levels of toxicant exposure, than either liversomatic index or histopathological changes (Dixon et al., 1987). However, an increase in SSDH levels has not been correlated with any meaningful changes at higher levels of organization.

Transaminase Enzymes

Serum levels of glutamic pyruvate transaminase (GPT) and glutamic oxaloacetate transaminase (GOT) levels in serum have been used widely in mammalian medicine as indicators of non-specific liver or kidney damage. These two transaminases, along with glutamate dehydrogenase, play an important role in the detoxification of ammonia (d'Appolonia and Anderson, 1980). In fish, the enzymes have also been used successfully to show impacts of toxicants (Weiser and Hinterleiner, 1980). Again, changes have not been correlated with alterations at higher levels of organization, and field work must carefully standardize collections. Furthermore, d'Appolonia and Anderson (1980) found that the contamination of reagent kits or blood samples with high levels of ammonia (or glutamate dehydrogenase) confounded interpretation. This observation is especially important since many types of liver damage also result in increased plasma ammonia levels. Other transaminase enzymes which have been utilized include serum aspartate aminotransferase and alanine aminotransferase (Versteeg and Giesy, 1986).

3.1.4.2.4 Microbial Indicators of Toxicant Stress

Bacterial Dehydrogenase Enzymes

Dehydrogenase enzyme activity is a crude indicator of heterotrophic activity. Dehydrogenases are an enzyme class which is a major representative of the enzymes which catalyze oxidation reactions by transferring electrons down the electron transport system. During incubations (generally less than 90 min), a redox-indicator dye is added to produce a colour change (Elnabarawy, 1986). Various dyes have been used, including methylene blue, triphenyl tetrazolium chloride (TTC), tetrazolium blue, resazurin and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-tetrazolium chloride (INT) (reviewed in Bitton, 1983; Bitton et al., 1986).

In general, heavy metals (Ag, Hg, Cr) are more toxic to dehydrogenase enzymes than organics (pulp mill effluent, phenol, formaldehyde) (Bitton, 1983). Measurement of dehydrogenase activity in sediments can be affected by several variables including oxygen conditions, sediment age, extraction procedure, incubation time and diluent type as well as sediment volume (Burton and Lanza, 1985). Dehydrogenase techniques do not correlate well with macrobioassays and are generally less sensitive than other microassays, such as Microtox (Munkittrick and Power, 1989). Some dehydrogenase enzymes, especially resazurin, show increased sensitivity to metals, complicating interpretation of field results.

Resazurin reduction: used to measure dehydrogenase activity of *Bacillus cereus* (Liu, 1985, 1986). Reagents are commercially available, and bacteria can be used from laboratory cultures or they can be isolated from the environment. Each bacterial species has its own sensitivity spectrum and, in general, the gram positive bacteria are more sensitive than the gram negatives (Liu, 1986). Liu (1985) used 6 different bacterial cultures and found that they all responded differently and that the dose response was not linear for most chemicals. Elnabarawy (1986) used activated sludge to measure resazurin reduction. The reaction is run at 21°C for 20 to 30 min and requires a spectrophotometer. The reaction is very stable, reproducible, can be performed within 2 h and has a coefficient of variation of 3% (Liu, 1986). The test has been validated for As^{+5} and HgCl (Liu, 1985).

TTC reduction: used to assess the dehydrogenase activity of activated sludge. The mixture is incubated at 37°C, pH 7.5 for 60 min and the results are read on a spectrophotometer. TTC is sensitive to cyanide (Slabbert and Grabow, 1986).

INT Activity: *Pseudomonas alcaligenes* is exposed to toxicant over a range of 4 to 5 concentrations at 28°C for 30 min with phosphate buffer (Walker, 1988). DMSO, ethanol and acetone have been used as solvents at concentrations of 3 to 6%, although enzyme activity in DMSO is generally less than in ethanol or acetone, and the assay is sensitive to pre-incubation and diluent type (Burton and Lanza, 1985; Walker, 1988).

Bacterial Fluorescence

Munkittrick and Power (1989) reviewed the impacts of metals on Microtox fluorescence. Intercomparisons were complicated due to the use of different exposure times, pH and assay temperatures. For some metals, the toxicity (as assessed by the Microtox assay) increases with temperature and a slow, progressive response is evident for most metals regardless of temperature (cf. Munkittrick and Power, 1989). Microtox was not as sensitive to inorganics as *Daphnia*. Microtox was also less sensitive than rainbow trout and fathead minnow, except to Hg, As and Co (Munkittrick and Power, 1989). In comparison with other bacterial assays, Microtox also showed a lower sensitivity to Pb, Cd and Ni. In interassay comparisons involving sediment extracts, Microtox was 875X less sensitive (based on differences in EC/LC50s; range 42-3000) than *Daphnia* to leachates involving electroplating sludge (Fe, Cd, Cr, Ni, Cu,; pH 4.4 - 9.1) (Calleja et al., 1986).

3.1.5 Biochemical Indicators of Growth

Growth is considered to be a good, non-specific, integrative measurement of an individual's response to contaminants and has been widely used (Dixon and Sprague, 1981b; Seim et al., 1984; Collvin, 1985; Munkittrick and Dixon, 1988a). Determination of the energy available for growth, based on the physiological analysis of the energy budget rather than the direct measurement of growth itself, is particularly useful in assessing the biological impact of pollution, since it provides an immediate assessment of the energy status of the animal as well as insight into the individual components which effect the changes in growth rate (Widdows, 1985a). Direct measurement of growth and production is difficult in many species because a large proportion of the total production can be lost in the form of gametes and/or because accurate measurement of tissue growth is confounded by the presence of a shell or exoskeleton. Various studies have described biochemical methods of estimating fish growth, including biochemical indicators of nutritional status, RNA/DNA rations, adenylate energy charge (ATP/ADP ratios), gene expression (mRNA production), glycine incorporations into scales, and oxygen to nitrogen ratios.

3.1.5.1 Biochemical Indicators of Algal Growth

Growth of algae may be measured directly by cell counts or biomass production (wet weight, dry weight, or ash free dry weight) of field collected specimens, or indirectly using fluorescence techniques that correlate their content of chlorophyll with their biomass. Devices for measuring fluorescence include a moored *in situ* fluorometer designed for plankton studies (Whitledge and Wirick, 1986; Cowles et al., 1989). Parameters measured include chlorophyll a fluorescence, temperature and conductivity. Both techniques provide measurements over a time period of weeks to months. Several biochemical methods can be used to evaluate growth of algae including the measurement of fluorescence or photosynthetic activity.

3.1.5.1.1 Fluorescence

The fluorescence emitted by plants and algae can also be remotely sensed and used to estimate biomass of aquatic plants and algae. Two types of applications are currently in operation: 1. passive ocean colour spectral analysis that uses the sun's rays to induce the fluorescence response, and 2. active or laser-induced fluorescence (LIF) that uses laser stimulation of fluorescence at 532 nm (green light). Such information is essential in determining global scale estimates of primary productivity and has application in evaluating carbon budgets of the oceans and lakes to address questions of global warming and the "greenhouse effect", as well as provide smaller scale information on issues such as eutrophication or changes in productivity of aquatic systems. Remotely sampled estimates of Chl a had high correlation (r values ranged from 0.82 to 0.97) with shipboard "ground truth" measurements of chlorophyll content (Walsh et al., 1988). By measuring fluorescence emission at two wavelengths, it is possible to determine the response of two types of photosynthetic pigments (phycoerythrin and Chl a) and, by implication, determine the concentrations of different components of the algal community (bluegreen and total algae, respectively).

Bristow and Nielson (1981) report a study of the use of LIF to estimate organic carbon in surface water of a large river system. Their results showed this method to be capable of measuring several water quality parameters simultaneously: Chl a, dissolved organic matter (DOM), and optical attenuation. Bristow et al. (1985) reported the application of the technique to the Columbia and Snake rivers. The use of LIF over such large contiguous surfaces is a marked improvement over traditional sampling techniques that rely on intermittent sampling over days (or weeks) from widely dispersed areas.

3.1.5.1.2 Photosynthesis

The use of enumeration techniques to determine growth of unicellular plants is a simple, but time-consuming technique. The use of more rapid, less labour-intensive technologies has distinct advantages, especially when testing rapidly changing effluents, such as those of municipal and/or industrial waste outfalls. Growth bioassays for plants have been adopted due to the difficulty of determining when a plant cell is "dead." Further explanation may be drawn by analogy with animal bioassays. If the biological endpoint of, for instance, a fish bioassay was death, the researcher could safely assume the mortality of the test organism if it stopped breathing for some extended period of time, rather than having to wait to see if the fish was able to grow or reproduce. The goal of a more rapid algal bioassay, then, is to evaluate the plant's "breathing." This can be accomplished by monitoring photosynthesis, as carbon dioxide uptake, oxygen evolution and/or fluorescence emission characteristics.

Carbon Dioxide Uptake Techniques

Carbon dioxide uptake techniques have been used successfully in algal studies and there exists a vast body of literature that supports their use. However, those techniques that require the use of radioactive material are not recommended for broad use because of the level of expertise necessary to perform the tests and the specific safety needs for radioactive waste handling and disposal. Other techniques, such as the use of infrared gas analyzers (IRGA) to monitor photosynthesis in vascular plants, have been used extensively and effectively in both laboratory and field experiments (Kaplan and Bjorkman, 1980; Powles and Bjorkman, 1982; Nobel and Hartsock, 1984; Nobel and Long, 1985; Geller and Nobel, 1987). This technique can evaluate sublethal effects of many toxicants before such obvious effects as chlorosis or necrosis occur.

Oxygen Evolution Techniques

Oxygen evolution is another measure of photosynthetic activity. This technique is used on aquatic plants and algae. Although oxygen electrodes are relatively inexpensive and simple to operate, they must be used in the laboratory, are fairly insensitive, and are subject to significant within-experiment variability.

Using a flow-through system for exposure of seagrass to pollutants, Walsh et al. (1982) measured the effect of PCP and atrazine on photosynthesis and respiration of *Thalassia testudinum*. The endpoints of this test were oxygen evolution by whole plants or leaves, measured after 40 or 88 h exposures to the toxicants, using an oxygen electrode. Results were expressed as oxygen concentration of the water (rather than on the more accepted and useful per-weight or per-Chl bases) and as changes in the photosynthesis to respiration (P/R) ratio.

Fluorescence as an Index of Productivity

A third technique that is both inexpensive and technically simple to master, can be performed in the laboratory or the field, and exploits an intrinsic quality of the photosynthetic apparatus of all plants, terrestrial and aquatic, is fluorescence. Fluorescence occurs in plants when excess light, absorbed by

Chl and other light-harvesting pigments, that cannot be used for photochemistry, is reradiated at a lower, specific energy level (wavelength). *In vivo* (protein-bound) Chl fluorescence of chloroplast membranes and algal cells is emitted, at room temperature, in the 650 to 800 nm (red) region of the spectrum. At physiologic temperatures, the vast majority of Chl fluorescence arises from Photosystem II (PSII) (Butler and Kitajima, 1975). The total amount of Chl a fluorescence has been correlated with content of Chl a. This technique is used as an indirect method for estimating biomass in algae (Cullen et al., 1986). While this is obviously an important use of the tool, the potential for application of this technique is much broader and can give information about the ongoing process of photosynthesis, not just about its end result, growth. Fluorescence appears to be a vital indicator of the "health" of the photosynthetic apparatus in terrestrial and aquatic test plants. Evaluation of certain fluorescence parameters has also been used successfully with algal systems to determine effects on photosynthesis. Fluorescence induction kinetics can also be used to screen for toxicant (e.g., herbicide-resistance) in algae because the shape of the fluorescence curve differs between these classes of organisms when exposed to the toxicant (Voss et al., 1984; Gressel, 1985; Neale and Richerson, 1987; Erickson et al., 1989).

3.1.5.2 Biochemical Indicators of Nutritional Status

Proteins, lipids, glycogen and sugars were measured in the mantle, digestive gland and gonads of clams at various times after transplantation to contaminated sites (Pellerin-Massicotte et al., 1989). A 30% decrease in sugars and lipids was observed after 30 d exposures to industrial effluents. Similar work has also been done on a variety of fish species exposed to acid mine waste. Munkittrick and Dixon (1988a) used muscle lipids, visceral lipid stores, liver glycogen, liver lipids, serum lipids, cholesterol and triglycerides to examine the impacts of mixed metal mining waste on the growth of white sucker. They found serum, muscle and visceral lipids to be the best indicators of growth status in those fish. Ranges of values for blood chemistry for several fish species are found in Lockhart and Metner (1984) and Hille (1982). Glycogen levels in the muscle and liver are dependent upon nutritional status and the recent history of the organism.

3.1.5.3 RNA and DNA Analysis

In invertebrates, total RNA content has also been used as an indicator for growth of *Daphnia magna*. Knowles and McKee (1989) found that, after exposure to pesticides, the RNA content of *Daphnia magna* was related to growth, and was a more sensitive indicator of impact than standard end points of survival and growth. However, the total RNA content cannot be easily applied to field studies, and cannot be used to predict the growth rates of organisms grown under different conditions. Knowles and McKee (1989) found that, under field situations, comparisons could be made if organisms were sorted by size and compared by regression analysis between sites.

Several investigators have suggested the use of protein, RNA and DNA relationships, and the ratio of RNA to DNA as bioindicators of reduced growth of aquatic organisms exposed to toxicants (Kearns and Atchinson, 1979; Barron and Adelman, 1984; Passino, 1984). Cd effects on NOEC for protein growth, RNA and DNA were compared to 21 d reproduction NOEC for *Daphnia*: there were differences in the Cd concentrations which induced measurable responses (Knowles and McKee, 1989). Cd effects on protein growth were most pronounced immediately after the rapid growth phase, whereas effects on RNA:protein, protein:RNA and protein:DNA ratios were most appropriately measured before the rapid growth phase. Barron and Adelman (1984) found that RNA content per larva was the measurement most highly correlated with early life stage growth.

3.1.5.4 Adenine Nucleotide Levels: Adenylate Energy Charge

Changes in the levels of adenine nucleotides in tissues can be used as nonspecific indicators of environmental stress. The basic premise is that measurement of the levels of adenine nucleotides will give some indication of the status of energy phase through an organism (NRCC, 1985). Stress, particulary toxicant stress, can be expected to increase the energy status of an organism either by increasing energy consumption for detoxification and repair, or by reducing energy production as a result of direct toxic action on oxidative metabolism or glycolysis.

The relative concentrations of ATP, ADP and AMP can be used to assess the availability of cellular energy (Heath, 1984). The major parameter which has been used to express energy impairment is the adenylate energy charge (AEC), which is the ratio of the charged portion of the adenylate pool to the total pool (a ratio of ATP/AMP+ADP+ATP). Changes are believed to be related to changes in cellular respiration or enzyme activity and, in laboratory studies, react prior to changes at higher levels of organization (Heath, 1984). The impact of modifying factors (temperature, fish size, handling stress, season, etc.) must be investigated. In addition, the optimal tissue for assay should be chosen. It is known that the components of the adenylate pool change after exercise (reviewed in Heath, 1984).

The utility of AEC in studies of microbial ecology is widely recognized (Karl, 1980; in NRCC, 1985). AEC levels in bivalves appear responsive to toxicant exposure (Ivanovici, 1980; Giesy et al., 1983; in NRCC, 1985), but the levels in crustaceans are relatively less responsive (Dickson et al., 1982).

Although AEC has been used in fisheries work to assess the impact of classical physiological stressors (e.g., oxygen, temperature, pH), there is little published work on the impact of toxicants. The value of the technique as a general measure of stress in the field depends on the number of stressors that affect the adenylate system.

While using AEC as an indicator of stress, it should be noted that AEC is not a measure of total energy available to an organism, but rather an indication of the degree of availability of that energy. AEC reflects the ability of an organism to regenerate high energy adenine nucleotides, but gives no indication of either the size of the actual adenylate pool or the size of the reserves used to regenerate those pools (glycogen, neutral lipids, creatine phosphate, arginine phosphate). Large changes in the size of the energy reserves and the adenylate pool can occur, but as long as the relative proportions of the three adenylates remain constant, AEC will not vary. Since AEC would appear to be the subject of rigorous homeostatic control (Atkinson, 1977), it may underestimate the impact of chronic stressors. Knowing the size of the actual adenylate pool may be of more value than knowing the specific AEC (Vetter and Hodson, 1984).

3.1.5.5 Gene Expression

Gene expression involves a chain of biochemical events starting with an hormonal stimulus at an hormone receptor site. This includes synthesis of messenger ribonucleic acid (mRNA) sequences in the appropriate gene of a deoxyribonucleic acid (DNA) molecule. The mRNA acts as a template for transfer (tRNA) and ribosomal RNA (rRNA). These forms of RNA decode the mRNA for the actual protein synthesis of ribosomes.

These reactions may be very sensitive to contaminant effects and may provide information on the molecular basis for toxicity. Gene translation in rainbow trout has recently been studied using techniques of molecular biology (Chen, 1983a). By cloning the genes coding for vitellogenin, the hepatically-derived egg yolk protein, radiolabelled RNA nucleotide sequences specific to vitellogenesis have been synthesized. Consequently, a bioassay has been devised where vitellogenesis is induced in immature trout by injection of estradiol (Chen, 1983a,b). The process and degree of gene translation are measured *in vitro* by excising the liver 48 h postinjection and incubating liver homogenate with the radiolabelled RNA-nucleotide sequences. If gene translation occurs, the labelled RNA sequences are incorporated stoichiometrically into completed mRNA molecules. These are isolated, purified and measured.

This bioassay is a promising research tool. Preliminary bioassays of injected ß-napthoflavone, a potent MFO inducer, show reduced vitellogenesis. The proposed cause was enhanced catabolism of estradiol by the MFO enzymes, such that the hormone stimulus was weakened. Similarly, in trout chronically fed dietary PCBs, the induction of vitellogenesis was reduced (Chen, 1983b). Chen (1983b) felt that more research was needed on the methods of exposure, due to leakage of both the toxicant and hormone after injection, and the methods for assay. At present, this bioassay is too complex and uncertain for application to ecosystem health assessment. In addition, there is little information about "normal" values, variability and modifying factors, and the test would not be easily applied in field situations.

3.1.5.6 Glycine Incorporation into Scales

The rate of deposition of scale material over long intervals of time has classically been used to estimate the growth rate of fish. Since collagen is the most abundant structural protein in fish scales, and since glycine is the major component of collagen, the rate of glycine incorporation by scales could theoretically be used as an estimate of growth rate (NRCC, 1985). Ottaway and Simkiss (1977a; in NRCC, 1985) have also proposed an *in vitro* method of quantifying biochemical growth rate by determining the rate of ^{14}C-glycine uptake by scales. Acclimation, scale incubation temperatures and handling have been shown to alter glycine uptake (NRCC, 1985). Since the minimum response to these types of stressors occurs between 30 and 60 min, removing the scales during the first 30 min of stress would eliminate this type of response (NRCC, 1985).

In work with feral bass (*Dicentrarchus labrax*), Ottaway and Simkiss (1979) found that glycine uptake was significantly higher for those populations that also exhibited higher back-calculated growth rates. Since growth is considered a meaningful integral of environmental stresses, biochemical measurements of growth could be useful, particularly as they are rapid and cost-effective (NRCC, 1985). For both carp (*Cyprinus carpio*; Goolish and Adelman, 1983a), and bluegills (*Lepomis macrochirus*; Goolish and Adelman, 1983b) a highly significant relationship between glycine uptake and growth rate for individual fish has been demonstrated (NRCC, 1985). Laboratory studies have shown that for both roach (*Rutilus rutilus*; Ottaway and Simkiss, 1977b) and bluegills (NRCC, 1985), mean glycine incorporation by scales of fed fish is significantly higher than that by scales of starved fish.

3.1.5.7 Oxygen to Nitrogen Ratios

A general response by an organism to stress is the utilization of nutrient reserves to meet a metabolic requirement that may have been elevated above normal values. This can be measured in terms of a depletion of carbohydrates, lipid and protein substrates, but it may be necessary to use a more sensitive index which reflects the alterations in the balance between the catabolism of these substrates. The ratio between oxygen consumed and nitrogen excreted (both components of the energy budget) provides an index of the relative utilization of protein in energy metabolism (reviewed in Widdows, 1985b). A high rate of protein relative to carbohydrate and lipid catabolism results in a low O:N ratio, which is generally indicative of a stressed condition. There are inter-specific and intra-specific differences in the ratios of "unstressed" organisms, depending upon trophic level and the nature of the nutrient reserves; therefore, comparisons should be based on relative values. This approach has been field validated (Widdows et al., 1981), but there is evidence of limitations, particularly during reproductive periods and for organisms with a protein-based metabolism, such as carnivorous invertebrates.

3.1.5.8 Yolk Absorption Efficiency

Since early life stages of fish are commonly the most sensitive stages to toxicants, several people have attempted to use yolk conversion efficiency as an indicator of the impact of toxicants on growth (Hodson and Blunt, 1986; Munkittrick and Dixon, 1988b). Trials commonly measure the proportion of body weight accounted for by yolk, at progressive stages of larval development. Toxicant impact may result in a decreased growth efficiency, as energy is drained to metabolize the contaminants, or an increased growth efficiency, as growth is stimulated by sublethal levels of exposure. It is important to evaluate both the rate of yolk utilization and the efficiency of utilization when examining the impacts of metals on the growth of fish larvae (Peterson et al., 1983).

3.1.6 Biochemical Indicators of Reproduction

Various biochemical indicators of reproductive status have been used, including serum steroid levels. Since the analysis of serum steroids requires a lot of expensive equipment, many studies use surrogate indicators of reproductive status. The most commonly used indicators for studies involving acidification and mixed metal impacts have been indirect indicators of vitellogenesis in female fish. These studies have been prompted by the observations surrounding impaired reproductive performance of fish in acid lakes.

Vitellogenin is a large lipophosphoprotein found in the blood of female fish, and represents the dominant form for transport of lipids into eggs. In addition to direct measurement of vitellogenin levels, studies also use measures of total serum Ca, serum bound Ca, and serum phosphoprotein to indicate changes associated with vitellogenesis (Parker and McKeown, 1987a; Tam et al., 1987; Mount et al., 1988a; Munkittrick and Dixon, 1988a). It now appears that most of the impacts of acidification, and the results of some metal studies, can be explained by changes in growth efficiency rather than direct reproductive impairment (Mount et al., 1988a,b; Munkittrick and Dixon, 1988a).

3.1.7 Cytological Changes

It should be possible to observe alterations in the structural-functional organization of individual target cells or groups of cells at an early stage of a reaction to cell injury, before an integrated cellular process would manifest at the level of the whole animal and long before community effects become evident (Moore, 1985). These changes include external alterations in the characteristics of protozoans, histological and ultrastructural changes in gill morphology in fish, ultrastructural indicators of lysosomal stability, and histological changes in various tissues, including interregnal, thyroid, and liver (Brown et al., 1984; Donaldson et al., 1984).

3.1.7.1 Ultrastructural Changes

Ultrastructural changes in the morphology of protozoans and diatoms have been described in response to exposure to metals and to acid stress. Cd exposure of the flagellated algae, *Euglena gracilis*, resulted in changes associated with numerous myelin-like structures in mitochondria, altered shape of chloroplasts and thylakoid arrangement and increase of osmiophilic plastoblobuli (Duret et al., 1986). Changes in the unicellular algae, *Poteriochromaonas malhemensis*, have been described after exposure to gasoline additives (Rõderer, 1981), including tetraethyl lead (Rõderer, 1986). The major impacts were on lorica formation in the chryosophyte, which is microtubule mediated. Rõderer (1986) concluded that lorica formation offers a practical and valuable test system for gaining information on interactions of toxicants with microtubules. Together with examination of mitosis and cytokinesis, it allows a look at the mechanisms of toxicity (Rõderer, 1986).

In *Cyclotella* (Bacillariophyceae), the short (2 h) and long term (8 h) effects of nutrients and Pb on ultrastructure were examined (Sicko-Goad et al., 1986). The authors found changes in the quantitative ultrastructure of the diatom, but cautioned that these effects were mediated by nutrients. It is clearly difficult to simulate the natural environment where a variety of parameters may be changing simultaneously. Sicko-Goad et al. (1986) advise that extra caution be taken in the interpretation of toxic threshold assays with natural populations, due to many possible interactions which make the work difficult to repeat.

3.1.7.2 Histological Impacts

Histological studies have been concerned with the study of a large number of tissues, but most work with metals and acid exposure has concentrated on fish gills. Johnson and Bergman (1984) found that gill lesions occurred in all organisms exposed at contaminant concentrations above a threshold level. Limitations and problems with respect to techniques and interpretation of histological studies are discussed by Johnson and Bergman (1984).

3.1.7.2.1 Gill Lamellar Changes

Disturbances in ionoregulatory processes in the gills have been implicated by laboratory studies to be the first of several direct physiological effects of acidification on fish (reviewed by MacDonald, 1983). Impacts of metals and acid on fish gills have examined a wide variety of changes, including hyperplasia (increased number of cells) and hypertrophy (increased cell size measured as cell height and volume) of mucous cells, separation of the basilar membrane, and necrosis and fusion of secondary lamellae (Mitz and Giesy, 1985; Versteeg and Giesy, 1986). Under extreme acid stress, the increased mucous layer which builds up on the gills may be a prime contributing factor to death (Fromm, 1980). In lakes with pH values of 5.2 and 5.8, the gills of fathead minnows exhibited hyperplastic primary lamellar epithelia, diminished respiratory lamellar surface areas, widened respiratory blood-water barriers and altered cellular composition and morphology (Leino et al., 1987).

While further work is required to increase sample sizes, the results of work by Leino et al. (1987) suggest that very soft waters with a low pH and toxic metal ions have deleterious effects on gills and the physiological processes associated with them. Histological evidence for a physiological effect on ionoregulatory mechanisms is provided by laboratory studies showing that chloride (ionoregulatory) cells in gills of fathead minnows react to chronic acid stress by proliferation and by changes in their morphology (Leino and McCormick, 1984; Karlsson-Norrgren et al., 1986). Youson and Neville (1987) felt that the accumulation of Al with and on epithelial cells, and increased exposure of chloride cells, reflected decreased oxygen uptake and increased ion loss in fish, and were probably responsible for the increased lethality of fish ion acidified environments.

3.1.7.2.2 Other tissues

Various histological changes have been examined in fish, including cell size (Weis et al., 1986), and intrahepatocytic granules (Lanno et al., 1987). A full review of the impacts of metals on liver tissue can be found in Gingerich (1982). Renal changes have also been addressed by Hawkins et al. (1980). Macrophage aggregate parameters in the liver and spleen were evaluated by Blazer et al. (1987) as a possible indicator of pollutant stress.

3.1.7.3 Indicators of Lysosomal Integrity

The lysosomal membrane is often a target of injury by contaminants and assessment of injury has been confirmed as a sensitive index of cellular condition (Moore, 1985). A number of methods, including lysosomal membrane fractionation and quantitative cytochemistry have been used to assess lysosome stability in aquatic macroinvertebrates. Lysosomal stability in digestive cells of the clam *Mytilus* is reduced in a dose dependent fashion by chronic exposure to crude oil, and endodermal lysosomes of the hydroid *Companularia flexuosa* show reduced stability as a result of Cu, Cd or Hg exposure (NRCC, 1985). In both laboratory and field studies with macroinvertebrates, a significant linear correlation has been demonstrated between lysosomal stability and scope-for-growth (Bayne et al., 1976, 1979). Supporting studies with fish are lacking.

3.1.8 Genetic Changes

Rapid screening tests for mutagenicity are used in an attempt to predict the potential for altering whole organism performance, through a decreased potential for survival or an increase in deformities (teratogenesis). Genetic impacts of metals have involved a number of tests, including chromosomal aberrations and sister chromatid exchange. A recent bacterial (microassay) test (cf. Section 3.1.8.3) has the potential for examining the impact of acid mine drainage. Other techniques include examining the electrophoretic patterns of serum proteins for genetic shifts. The shifts in protein production presumably reflect changes in the genetic patterns of populations in response to pollutant stress (cf. Section 3.1.4.2.1, although any proteins can be used).

3.1.8.1 Chromosomal Aberrations

Chromosomal aberrations can arise as a result of physical or chemical impact on genetic material, and can take the form of either direct damage to chromosomes or structural alterations (mutations) in DNA. Increased incidence of mutations in a population is generally considered indicative of a reduced adaptive capacity (NRCC, 1985). The test is labour-intensive and subjective, requiring a determination of the incidence of metaphase cell chromosomal aberrations in tissue smears. Both *in vitro* and *in vivo* laboratory studies with biological tissue from several levels of taxonomic organization have demonstrated that mutagen exposure can result in elevated numbers of chromosomal aberrations in metaphase cells (NRCC, 1985). Weis and Weis (1989) showed that methyl mercury produced cytogenetic effects, including decreased mitotic count and increased frequency of chromosomal aberrations. Little or no information is available about the influence of modifying factors on chromosomal aberrations in natural populations.

Few field studies have demonstrated a correlation between the incidence of chromosomal aberrations and toxicant exposure. The incidence of chromosomal abnormalities in eggs of several marine species has been increased after exposure to fuel oil and complex mixtures of contaminants (reviewed in NRCC, 1985).

3.1.8.2 Sister Chromatid Exchange

Sister chromatid exchange is a sensitive indicator of chromosomal damage and is based on the switching of labelled arm segments within chromosomes (Moore, 1985). This cytological technique involves an examination of chromosomal abnormalities during cell division, and involves recording the incidence of abnormal splitting of chromosomes. The test has not been widely used and has not been correlated with changes at higher levels of organization (population, community).

3.1.8.3 Bacterial Indicators of Genotoxicity

3.1.8.3.1 SOS-Chromotest

The SOS-Chromotest is a new genotoxicity test described by Dutka et al. (1986) and Xu et al. (1987). This assay uses colorimetric indicators for activity of the beta-galactosidase (BGSD) enzyme system as an indicator of toxicant impact. The strain of *E. coli* used (K12-PQ37) has been genetically altered so that the galactosidase enzyme acts as an indicator of activity of cellular DNA repair mechanisms. The SOS-repair system is induced after DNA damage and, in this strain of bacteria, one of the control genes for the SOS system (sfia) has been fused to an operon which acts to turn-on the BGSD enzyme (lac-z) when the SOS-system is activated (Quillardet et al., 1982). The test therefore provides an indication of chemical pollution which may have genotoxic or mutagenic effects (Dutka, 1988), and the BGSD activity acts as a marker for genotoxic impacts. To distinguish between toxicants which kill the bacteria and those causing DNA damage, the assay also incorporates simultaneous determinations of alkaline phosphatase (AP) activity. Positive recording of AP activity ensures that the bacteria are still viable during the test.

The assay measures the SOS-inducing potential and can be used with rat liver microsomal mixtures (S9) similar to the Ames test. The S9 mixture is used to simulate the liver metabolic capabilities of mammals, and the microsomal fraction contains enzymes which convert the toxicants into forms which would be found in mammalian systems after exposure to the metabolic pathways in mammalian livers. The SOS-Chromotest was recently developed and therefore has not been widely applied.

3.2 INDIVIDUAL INTEGRATORS

The average values for all of the individual effects discussed in the preceding sections provide information at the primary level of response for the population level of biological organization. The organismic level of biological organization is a reasonable compromise in sensitivity and ecological interpretation, relative to the biochemical/cellular level and the population and community levels. Physiological responses to environmental degradation and their integration by organisms include responses such as changes in histology and morphology, growth, behaviour, reproductive potential and survival.

3.2.1 Morphological Changes

Histological and morphological changes in the tissues of fish and crustaceans have been recorded after exposure to a variety of metals during laboratory testing. Early observations showed a high correlation between indices describing histological and morphological changes in individuals and elevated levels of contaminants in field situations. Although these changes are assumed to be the result of altered enzyme function(s), little evidence has been available to directly link histological and morphological changes with contaminant levels or changes at the level of the population or community.

In some cases, it has been possible to link the development of neoplasms with contaminated sediments (Malins et al., 1985, 1988). Many of the chemicals accumulated in bottom dwelling fish are linked to "diseases" and health problems (e.g., liver carcinomas) in fish (Malins et al., 1984). However, few studies have attempted to link neoplastic changes in fish to changes at the population or community level. The link between morphological and histological changes in contaminant exposed populations has been made (Malins et al., 1988); what remains is to make the link between these changes and sublethal and/or chronic effects. A wide variety of morphological changes have been addressed, including skeletal anomalies, tissue somatic indices, tissue deformities (teratogenesis) and neoplasm incidence.

3.2.1.1 Skeletal Anomalies

A variety of factors can lead to damage to the spinal column of fish, including congenital defects (some induced by contaminants), parasitic infections, electrical shock and direct or indirect contaminant toxicity (NRCC, 1985). Skeletal anomalies include deformed fins, the lack of one or more fins and pelvic girdle, pugheadedness, asymmetric cranium, shortened operculae, fused and deformed vertebrae and spinal curvatures (Slooff, 1982). Besides congenital defects, there are three basic mechanisms of spinal damage (NRCC, 1985):

1. Spinal neuropathology: The destruction of spinal neurons which induces a loss of balanced bilateral muscle tone and causes the fish to bend.

2. Auto-fracture: Sudden violent muscle spasms induced by shock or fright can dislocate vertebrae and cause spinal curvature. Contaminants affecting the kidney can induce loss of ion regulation which may cause excessive muscle contraction and spinal curvatures.

3. Interference with collagen metabolism: Collagen formation, including both the incorporation of amino acids into collagen molecules and/or calcium, magnesium and phosphorus into the collagen matrix, may affect the strength of the vertebral column. This may make the fish more susceptible to auto-fracture.

The symptoms of spinal curvature are shown by external evidence of curvature, X-rays, biochemical measurements of collagen and reduced mechanical strength (NRCC, 1985). In areas contaminated with high levels of organochlorines and metals, the collagen levels in fish has been lowered, and the bone strength reduced (Passino, 1984). Baumann and Hamilton (1984) hypothesized that a high rate of spinal deformities in fish may be related to a short-term exposure to organophosphate pesticides, causing muscular contractions and resulting vertebral damage.

It is important to separate congenital-induced spinal deformities from contaminant-induced deformities, and age distribution of prevalence may help in this regard (NRCC, 1985). Contaminant-induced deformities increase in frequency with age due to increasing time of exposure. Modifying factors include species, fish age, vertebral size and adverse environmental conditions (temperature, light, low oxygen). Fish cannot be collected by electroshocking, as this may induce auto-fractures.

Spinal pathology has been advocated as a useful indicator of contaminant effects on fish because the prevalence of spinal curvatures in fish often increases with increasing proximity to pollution from urban and industrial developments (Bengtsson, 1983) and spinal curvatures have been induced during chronic bioassays for chemicals (reviewed in NRCC, 1985). Field studies by Mehrle et al. (1982) have demonstrated a strong correlation between measures of vertebral strength in U.S. east coast striped bass and tissue levels of organochlorine compounds, proximity to sources of pollution, prevalence of spinal curvature, and reductions in population size.

3.2.1.2 Teratogenesis

Metals can interfere with development by disrupting metabolic processes. If this interruption occurs during critical developmental periods, the metals can act as teratogens (Daye and Garside, 1980; Weis and Weis, 1987). Such interruption can occur through disruption of mitosis, interference with translation or transcription, metabolic disturbances in energy utilization or nutritional deficits (reviewed in Weis and Weis, 1987). Deformations can involve the eyes, heart, skeleton, pigmentation, and have been associated with exposure to several metals, including Pb, Hg and Cd (Weis and Weis, 1987). Munkittrick and Dixon (1989c) found that the patterns of deformities common in sites impacted by acid mine drainage were different from those found in heavily industrialized basins, indicating that both the frequency and type of deformity may have some value for monitoring programs (Dixon and Munkittrick, 1988). Similar observations have been made for invertebrate species, as well as fish.

For larval bluegill, Woock et al. (1987) demonstrated that elevated dietary Se exposure to parent fish causes teratogenesis and decreases larval survival. Problems such as edema, lordosis, scoliosis, kyphosis and jaw gape were observed in the larvae that hatched but did not swim up; less than 5% of the larvae that survived to swim-up had deformities. Scudder et al. (1988) reported that deformity rates in Cu-exposed fathead minnows were a more sensitive indicator than survival or growth.

3.2.1.3 Meristic Asymmetry

Some investigators have attempted to use asymmetry in meristic characteristics (numbers of fin rays, pores, branchiostegal rays, etc) to examine the impacts of acid and metal stress during early developmental stages of fish (e.g., Jagoe and Haines, 1985). The characteristics best showing asymmetry varied between species although the correlations were very weak. The weak relationships, a requirement for large sample sizes, and the lack of a defined cause and effect relationship limits the suitability of this technique (Jagoe and Haines, 1985). In their investigation of the impacts of acid mine waste on white sucker populations, Dixon and Munkittrick (1988) did not find any evidence of asymmetry in a small number of meristic characteristics examined (Munkittrick, unpubl. data).

3.2.1.4 Tissue Somatic Indices

Metabolic responses of fish to contaminant exposure often result in swelling or atrophy of specific tissues. For example, liver enlargements are due to a high carbohydrate diet (Dixon and Hilton, 1981) or increased enzyme activity for detoxification of compounds (Dixon et al., 1987). The spleen will shrink during haemolytic anaemia as dying red blood cells are rapidly replaced from splenic stores (Lanno and Dixon, 1989). Gonadal atrophy, which prevents maturation and reproduction, can arise from food limitation or reproductive dysfunction.

These effects may be detected by expressing tissue weights as a proportion of the total body weight to give a Tissue Somatic Index (TSI), such as the hepatosomatic index (HSI, also called liver somatic index) or the gonadosomatic index (GSI). TSIs do not have a specific diagnostic value since they respond to many factors. Nonetheless, they provide an early indication of pathology or enhanced metabolic activity. As such, TSIs are a useful screening tool to limit more expensive alternative tests. TSIs will be affected by sexual development, as the proportion of gonadal tissues changes with the stage of maturation. Also, TSIs should be considered to be species specific, as there are wide differences in body forms in fishes.

TSIs are determined by weighing both the whole fish and each tissue of interest as accurately as possible; no special equipment or expertise is required. The greatest source of error will be tissue water content. Fish held for extended time periods in gill nets, starved for long periods, or exposed to nephrotoxins will retain or lose water (NRCC, 1985). However, the bias may be equal for both tissue and whole body weights. Since gonadosomatic indices (GSIs) can be related directly to fecundity or reproduction, their relevance is easily understood. However, other somatic indices are only indirectly related to specific tissue pathology and effects on organism health. The real value of TSIs is as a surveillance tool for managers, when they are used in combination with other biomonitors.

3.2.1.5 Lesions and Neoplasms

Cell proliferation or tumor formation can increase the size of specific tissues into an enlarged area (neoplasm) (Sonstegard and Leatherland, 1984). Increases in the numbers of neoplasms and lesions have been reported in highly contaminated regions for a number of years (Larsson et al., 1985; Overstreet, 1988). The use of pathological indicators in pollution assessment and monitoring includes changes in melano-macrophage aggregates in fish. A relationship of environmental stressors, including pollution and disease, to increases in number, size and pigment content of these aggregates has been suggested in several studies (Sindermann, 1988). However, little effort has been made to correlate these with changes at the population or community level (Munkittrick and Dixon, 1989b). Studies of the comparative epizootiology of tumours in fish have disclosed numerous examples of an association of high tumor frequencies with polluted habitats (reviewed in Sindermann, 1988). It has been suggested that high prevalence of such tumours can be used as a "sentinel" of environmental danger. Reproductive tumors have been reported in fish near metal mining sites, although there has been concern that these were not related to the metals *per se* (Black et al., 1982).

3.2.2 Behaviourial Changes

It is clear that organisms can and do respond to contaminants by altering their behaviour. Basic behaviourial patterns (e.g., locomotion and orientation) are essential to processes such as prey capture, feeding, predator avoidance movement, migration, courtship and mating. The integration of these behaviours will, in part, determine the success of each individual and of the population. Behaviourial responses to contaminants in aquatic systems include a wide range of behaviour such as avoidance, inhibited feeding, increased random movement and other behaviours (Sprague, 1976; Fromm, 1980; Steele et al., 1985; Lemly and Smith, 1987).

A behaviourial response is an integration of physiological responses to a chemical stimulus. Chemoreception in fish is believed to play a mediating role in reproductive migration and pairing, schooling, feeding, parental recognition and predator avoidance (Hara, 1982). Contaminants that affect the normal function of neurosensory systems may affect how organisms move through their environment and respond to the normal range of cues that direct them toward food, shelter, spawning grounds and other necessities for population growth. Various behaviourial endpoints have been addressed, including spatial selection, response to food and feeding ability, predator-prey responses, aggression, displays, reproductive behaviours, ventilatory and cough responses and preference or avoidance to a variety of stimuli.

3.2.2.1 Ventilatory/Cough Responses

Atchison et al. (1987) found that ventilatory and cough responses were among the most sensitive behaviourial responses to metals. These responses are usually measured electronically, and threshold response levels vary widely between species. Exposure of rainbow trout to Al under varying pH conditions caused increased activity responses and ventilation rates ranging from slight to severe depending upon the pH (Neville, 1985). Using laboratory data collected under a wide range of pH conditions for fish responses to Al, Neville (1985) predicted possible detection and avoidance or survival, assuming increased activity (and therefore use of energy) in the field compared to the testing conditions in the laboratory. The ecological consequences of increased cough rates are unknown, but may result in increased aggression (Atchison et al., 1987). Increased ventilatory rates may result in an increased rate of toxicant uptake, as increased water flow over the gills may result in an increased exposure of gill tissue to waterborne toxicants.

3.2.2.2 Preference/Avoidance Responses

In mayflies, an increase in drifting behaviour has been associated with exposure to decreased pH and elevated metals (Raddum and Fjellheim, 1987). Avoidance behaviour has been linked to chemoreception in fish by experiments which determined the toxicities of Hg, Cu and Zn to olfaction in coho salmon (*Oncorhynchus kisutch*) (Rehnberg and Schreck, 1986). Also, the effect of metals on binding of L-serine (a model odour) with the olfactory cell membrane receptor was studied. It was found that the detection of L-serine was inhibited by Hg and Cu, but not by Zn. None of these metals interfered with serine detection by forming non-stimulatory metal-serine complexes. All three metals were found to disrupt simple upstream movements in an experimental apparatus (Rehnberg and Schreck, 1986).

Fathead minnows (*Pimephales promelas*) preferred elevated concentrations equal to three times the holding exposure concentration after a three month pre-exposure to a mixture of Cu, Cr, As and Se (Hartwell et al., 1987a,b). After 6 months of exposure, the fathead minnows mildly avoided concentrations 5 times the holding concentration and were not responsive to concentrations approaching 10 times the holding exposure level after 9 months of acclimation (Hartwell et al., 1987). This response strongly suggests that there is a long term acclimation by fish to metals and that the rate and/or degree of acclimation is time dependent. This work was followed up with field validation (Hartwell et al., 1988).

Atchison et al. (1987) concluded that locomotory responses could be the result of several behaviourial changes, including attraction, avoidance, altered sensory perception which reduces the responses to normal olfactory cues, induction of hyper- (or hypo-) activity, and reduced swimming performance.

3.2.2.3 Reproductive Behaviour

Low levels of Cd have been shown to affect the competitive ability of male isopods and amphipods to search for reproductive females, and to result in altered precopulatory behaviour (Poulton and Pascoe, 1989). Some metals have been shown to accumulate in the gonads of fish with subsequent reproductive effects (Baumann and Gillespie, 1986; cited in Pyron and Beitinger, 1989), Pyron and Beitinger (1989) found that fathead minnows exposed to high concentrations of Se demonstrated typical reproductive behaviours, which resulted in gamete release and fertilization. However, the offspring tended to have edema and low survival rates. Impacts of metals on courtship behaviour and nest-building were reported in a review of reproductive behaviours by Atchison et al. (1987).

3.2.2.4 Feeding Responses

With respect to acid mine waste, a large number of behaviourial studies examining feeding responses have been conducted. In preference-avoidance studies, a metal mixture (Cu, Cr, As and Se) affected the response of crayfish to a feeding stimulant (Tetramin filtrate) (Taylor et al., 1989). No responses were contaminant-specific, but for acidification and some metals (notably Al), the feeding response of salmonids was a more sensitive indicator of Al exposure than was mortality, growth, whole body ion content or RNA/DNA ratios (Little et al., 1989). Reduction of pH to approximately 6.0 impaired feeding behaviour and reduced food intake, fecundity and long term survival of fathead minnow populations (Lemly and Smith, 1987). Work with juvenile largemouth bass (*Micropterus salmoides*) showed that exposure to low pH (3.7) reduced time spent feeding, while juveniles at pH 6.1 and 4.8 performed more feeding acts and spent more time feeding than bass held at pH 7.2 (Orsatti and Colgan, 1987).

Lemly and Smith (1985; 1987; both cited in Smith and Lawrence, 1988) found that the searching response of fathead minnows to chemical feeding stimuli decreased when the pH of water went below 6. Impacts have also been described on aggressiveness during feeding, prey detection and capture, reaction time, foraging behaviour, predator avoidance (reviewed in Atchison et al., 1987). Gunn and Noakes (1987) found that lake trout alevins exposed to decreased pH and increased Al were less efficient predators.

3.2.2.5 Other Behaviourial Responses

Behaviourial examinations of invertebrates have used a number of sensitive tests, including movement, feeding and reproductive responses. The righting reflex latency has been used with gastropods to examine the impact of contaminants, and retardation of the righting reflex has been associated with foot paralysis after exposure to PCBs (Jahan-Parwar, 1989). Smith and Lawrence (1988) investigated the effects of low pH on the response of fathead minnows to a chemical social signal, a pheromone alarm substance. However, acute exposure of adult fathead minnows to pH levels ranging from 8.0 to 5.0 had no significant effects on their behaviourial responses to alarm substance.

3.2.3 Growth

Growth is a fundamental component of fitness, and therefore is an important index of contaminant effects. Toxicants can affect growth rates indirectly by reducing the food available, and directly by impairing metabolic pathways converting food energy to tissue or by diverting energy from growth to metabolism of the contaminant. Effects on growth (and reproduction) can best be understood by considering the energy budget of an animal (Widdows, 1985a). Food energy consumed is used for respiration and production of tissue or gametes. There is also some loss via the faeces and excretion. When production is estimated from the difference between the energy absorbed and the energy expenditure via respiration and excretion, it is referred to as the "scope for growth" (Warren and Davis, 1967; cited in Widdows, 1985a). Scope for growth can range from positive values when there is energy available for growth and the production of gametes, to negative values when the organism is utilizing its body reserves for maintenance metabolism. The concept of scope for growth has been widely used to assess the sublethal biological effects of a range of toxicants in marine invertebrates (Widdows et al., 1985a). Time series determinations can assess the severity of energy deficiency and can detect any changes in rates as well (Sindermann, 1988); field validation experiments support the use of scope for growth as a bioindicator of environmental stress (Widdows et al., 1984).

An additional index can be calculated from the physiological components of the energy allocation to provide further information on the efficiency with which an animal functions. The energy available for growth, as a proportion of the energy absorbed from the food, represents net growth efficiency and is a measure of the efficiency with which food is converted into body tissue. A reduction in this value is indicative of a stressed condition, since a greater proportion of the energy absorbed from the food is being used to maintain the animal and consequently a smaller proportion is available for growth. In a field study, Widdows et al. (1981) showed that increasing pollution (measured as increased tissue concentrations) was correlated with a reduction in net growth efficiency.

The growth of the mayfly, *Isonychia bicolor*, was measured as the number of moults over time, and was shown to be a sensitive indicator of the effects of Ag (Diamond et al., 1989). There are important differences between laboratory and field studies of toxicant impacts. With fish, field studies of the *in situ* toxicity of acidified surface waters have found that survival was a better measurement of toxicity than growth due to the presence of confounding factors (Parkhurst et al., 1989).

Exposure of fish to metals has been associated with decreased growth (Lett et al., 1976; Dixon and Sprague, 1981a,b; Seim et al., 1984; Collvin, 1985; reviewed in Munkittrick and Dixon, 1988a). An initial decrease in growth has been associated with the synthesis of protective proteins, such as metallothioneins (Roch et al., 1982; Bradley et al., 1985) and to decreased appetite (Lett et al., 1976). Although acclimation of growth may be slow (Collvin, 1985), growth rates usually return to normal or surpass those observed in reference groups (reviewed in Munkittrick and Dixon, 1988).

Measurements of scope for growth (SFG) and net growth efficiency (NGE) have been developed. SFG offers an instantaneous view of sublethal effects which, if extended over a period of time, would result in death. NGE values provided a long term integration of physiological processes. Growth is viewed as a good integrative measurement of an individual's response to contaminants and has been widely used. It was concluded that analogous exposures and exposure-response relationships developed in the laboratory were not different than those in the field. The consequences of reduced growth include reduced fecundity, slower maturation (Munkittrick and Dixon, 1989a) and a reduced ability to compete with other individuals: these consequences have population and community level repercussions.

3.2.4 Reproduction

Contaminants can affect reproductive processes in several ways, including altering the availability of energy, metabolic disruption of factors affecting reproductive control, impacts on reproductive behaviour and changes in reproductive performance. Energy allocation can be affected by decreasing the amount of energy available for reproduction through food limitation or through the metabolic utilization of energy reserves for dealing with contaminant burdens (Munkittrick and Dixon, 1988a). Toxicological experiments on reproduction of species with a short life span have been described as the most productive for useful results (Sprague, 1976). This parameter is of ecological importance because it has a direct influence on recruitment and the maintenance of a population.

In invertebrates, similar perturbations in reproductive processes occur, but less work has been done on the response physiology/biochemistry; most of the papers reviewed were bioassay oriented with the endpoints being measures of reproductive processes or success. These included delays in sexual maturation, delays in brood release, egg development time, brood size, frequency of reproduction and complete inhibition of reproduction. The repercussions of these reproductive effects are seen at the population and community levels which integrate all of the processes discussed here.

A pH of 4.0 had very little or no effect on the hatching success and reproduction of several species of Ephemeroptera and Odonata (Berrill et al., 1987) or several species of molluscs (Servos and Mackie, 1986). Mackie (1987) felt that neither decreased pH nor elevated metals were sufficient to result in lethality of freshwater Mollusca. In contrast, hatching success was reduced in frogs (*Bufo americanus* and *Rana sylvatica*) at low pH and was further reduced by the addition of Al. Egg mortality due to hydrogen ion stress was correlated with a delay in time of hatch and a reduction in egg size; however, there was no consistent pattern with respect to Al stress. Tadpoles were less sensitive than eggs, showing no response to Al or pH at the concentrations tested (Clark and LaZerte, 1985).

Effects measured in fish also include alterations in hatching frequency and hatching rate of eggs, and the survival and stress tolerance of embryos and larvae (Landner et al., 1985), spermatozoan motility (Mohr and Chalanchuk, 1985; Khan and Weis, 1986) egg yolk conversion efficiency (cf. Section 3.1.5.7), egg size (Shephard, 1987; Munkittrick and Dixon, 1989b), larval size (Munkittrick and Dixon, 1988b, 1989b), gonadal size and fecundity (Trippel and Harvey, 1987; Ram and Joy, 1988; Munkittrick and Dixon, 1988a). With some contaminants, impairment of reproductive success can be induced by exposure of adult fish to concentrations of contaminants as least five times lower than those yielding effects during direct exposure to embryos and larvae (Landner et al., 1985). Similar increased sensitivity has been shown for combinations of metals, where mixtures of Cd-Hg, Cd-Zn and Zn-Hg all showed significant reductions in reproductive success of *Daphnia magna* at concentrations where the single metals alone caused no significant effect (Biesinger et al., 1986).

Sensitivity of the larvae to metals can vary with developmental state (Meisner and Hum, 1987; Michibata et al., 1987; Dixon and Munkittrick, 1988; Munkittrick and Dixon, 1988b, 1989c). Responses of egg and fry stages of six fish species to low pH and Al were examined in a series of continuous flow toxicity tests using concentrations of toxicants and major ions (Holtze and Hutchinson, 1989). Low pH was lethal to cleavage eggs in the first 4 d of exposure, to eyed eggs in the immediate pre-hatch period and to fry following their transition to branchial respiration. Sensitivity to low pH and Al (for survival of early life stages) also varied widely between native species of fish, although laboratory results corroborated field evidence based on the presence and absence of species (Holtze and Hutchinson, 1989).

It is widely recognized that hardness contributes to a reduction in toxicity, but there is disagreement over whether Ca or Mg ions contribute to this phenomena. Mitchibata et al. (1986) examined the effects of these ions on the toxicity of Cd to the eggs of the teleost, *Oryzias latipes*. It was found that Ca contributed to the suppression of Cd toxicity, while Mg ions did not.

3.2.5 Genetics

Although aquatic organisms commonly demonstrate an increased tolerance to metals after preexposure (cf. Section 3.2.5), there has been little evidence for the vertical transmission (maternal to offspring) of tolerance in fish (reviewed in Klerks and Weis, 1987). However, Munkittrick and Dixon (1988b, 1989c) found that larvae from the AMD-contaminated sites exhibited a twofold increase in copper tolerance relative to controls (Munkittrick and Dixon, 1988a). This response was evident even though the eggs had not been exposed to elevated metal levels during either fertilization or incubation, but disappeared after the completion of yolk resorption.

3.2.6 Survival

Mortality at the individual level can be described as direct (acute) or delayed (chronic). In the field of toxicology the term "survival" has the connotation of acute lethality during a short term bioassay test. The term "bioassay" refers to types of laboratory tests in which one (or several) organism(s) is (are) exposed to a water sample (or concentrations of a known toxicant) for a defined period of time and a biological endpoint (e.g., survival) is measured. It is widely recognized that waters which are not acutely toxic may exert chronic toxicity. The most useful information relating to acid mine drainage impacts would be field data on survival of individuals residing in a contaminated habitat over an extended time period. Without marking individuals in a population, it is difficult to measure individual survival rates; therefore, the solution has been to measure survival in the laboratory in short term experiments, or to use caged fish studies.

When considering the potential effects of toxic chemicals (or other stressors) on individuals, populations and communities, it must be recognized that organisms are capable of adaptation to toxic conditions. Adaptation to chemical exposure can take several forms, including the altering of reproductive strategies or the development of acclimation (enhanced tolerance or resistance after first exposure). There are conflicting uses of the terms adaptation, acclimation, acclimatization, tolerance, resistance and hormesis in the literature. The following definitions are modified from those found in NRCC (1985) and Klaverkamp and Duncan (1987).

Adaptation is defined as the capacity of a species to change, and depends on the ability of the individual or the species to withstand environmental changes, and the genetic variability of the population. Adaptation can be either genetic, occurring within populations over time, or physiological, occurring within an individual during its lifetime.

Acclimation is a widely-described phenomenon evident in populations of organisms from bacteria to fish. Acclimation and *acclimatization* are often used interchangeably within the biological literature to mean changes in morphology and physiology of an organism in response to environmental change. However, environmental physiologists generally restrict the use of the terms to somewhat different compensatory changes. Acclimation includes those modifications acquired by an organism in response to experimental manipulation of a single environmental factor. Acclimation to metal toxicity may be produced by one of several processes, including decreased uptake, increased excretion, redistribution to less sensitive target organs and induced synthesis of metal binding proteins, or by some combination of these processes. When several factors vary simultaneously, it is difficult to determine which requires adaptation, and the word acclimatization is used. Acclimatization is often used to refer only to changes occurring within the natural environment.

Tolerance and *resistance* can be defined as the ability of an organism to exhibit decreased response to a chemical relative to those shown on a prior occasion. Tolerance implies that the change is within the normal adaptive range of the organism and can be sustained indefinitely (a real, adaptive change in LC50). Resistance implies that the magnitude of the factor lies outside the normal range and that detrimental effects will eventually ensue (only a change in LT50 results). Resistance has also been defined as an organism's ability to survive for a limited period in an environment that will eventually exert a lethal effect and is expressed as changes in survival times in lethal toxicant concentrations.

Adaptation also includes *hormesis* (hormensis), which is a wide-spread, poorly described phenomenon whereby organisms show a stimulation of performance elicited by a sublethal (sub-threshold) exposure to a stressor (Stebbing, 1982). When this phenomenon was first observed, it was called the Arndt-Schulz Law or Hueppe's Rule, because it was thought to occur generally. It is not yet clear if these types of responses are examples of successful adaptation, or represent unsuccessful adaptation, with as of yet undescribed low-level, long-term detrimental impacts.

Subtle differences in water quality can affect the behaviour, activity and bioavailability of chemicals. *Modifying factors* are defined as "any characteristic of an organism or the surrounding water which affects toxicity" (Sprague, 1985) and are usually divided into two descriptive groupings, biotic (intrinsic) and abiotic (extrinsic). Modifying factors can act to either increase or decrease the concentration of a chemical required to produce a biological response and the impact can vary dramatically between classes of chemicals and the organisms which are exposed. A biological response is detectable when the chemical reaches a sufficient concentration at the target site to affect the measurable performance of the organism. Threshold concentrations vary between chemicals and organisms, and modifying factors alter the rate at which chemicals reach the target site by changing the availability of the chemical to the organism, or the internal transport rate at which the chemical reaches the target site. The target site can vary with the concentration of chemical affecting the organism.

Both abiotic and biotic modifying factors affect toxicity by altering the external concentration of toxicant required to achieve the threshold internal concentration at that target site, for that chemical, at that dose, and for that organism. Factors affecting chemical activity can interact either within the organism or externally. Internal factors are usually biotic and act to change the manner in which organisms deal with a chemical metabolically. By increasing the rate of metabolic breakdown or excretion rate of a chemical, the dose (exposure) required to achieve the threshold concentration at the target site increases. External factors are usually abiotic, and affect the availability of the chemical for uptake. Chemicals, particularly metals, respond to some modifying factors by changing their speciation state, and some chemical species are able to reach target sites faster than others by crossing membranes more quickly or through preferential uptake by active mechanisms.

Examples of biotic modifying factors include species, life stage, sex, reproductive state and nutritional status, body size, diet and acclimation. Abiotic modifying factors of metal toxicity include water hardness, alkalinity, humic acid, dissolved oxygen, chelating agents, suspended solids, amino acids, and the presence of organic matter. The literature could not be and was not thoroughly evaluated based on these parameters; thorough reviews are available elsewhere (Sprague, 1985). Due to the episodic nature of rainfall, the survival of organisms in a system subject to acid mine drainage will depend not only on their tolerance to the magnitude of the pH depression, but also on their resistance to the duration of the perturbation. In addition, tolerance and resistance may vary over the life cycle and among animals from different populations depending upon their history of exposure. Both tolerance and resistance to metals and pH vary with a large number of biotic and abiotic modifying factors.

Several single-species algal toxicity test protocols have been developed to evaluate the toxicity of various types of effluents (Walsh, 1988a,b). Various endpoints are used including the number of cells at the beginning and end of the exposure period (cell count) and the number of dead cells (evaluated by using a differential staining response; Walsh, 1983). Similar types of studies have included ash free dry weight and chlorophyll concentration (Yount and Richter, 1986).

Recent literature includes descriptions of the impacts of differences with species (Nebeker et al., 1986a,b; Spehar and Fiandt, 1986), pre-exposure (Klaverkamp and Duncan, 1987; Hartwell et al., 1988), genetic strains (Orciari, 1979; Schom, 1986; Frauce and Stokes, 1987) and the duration and characteristics of exposure (Siddens et al., 1986). Siddens et al. (1986) demonstrated that repetitive, intermittent exposures to Al under acidic conditions produced greater cumulative mortality and lower growth rates of brook trout (*Salvelinus fontinalis*) than would be expected from continuous exposure. A summary of effects of pH changes on fish is provided in Table 3.

Studies on fish demonstrated increased tolerance, based on physiological adaptations to As and cyanide, Cd, Cu, blends of heavy metals, detergents and Zn (reviewed in NRCC, 1985). The response of fathead minnows which had been acclimated to a metals blend (Cu, Cr, As and Se) for three months was completely different from that of control fish (Hartwell et al., 1988). Acclimated fish did not respond to elevated metals levels in either artificial stream or field tests and the increase in resistance did not match the degree of loss of behaviourial responsiveness (avoidance) to metals.

Although it has yet to be used, measurement of tolerance could be applied to environmental health assessment. Luoma (1977) suggests that if one population of a species is more tolerant of a toxicant than others, this constitutes strong evidence that the toxicant at the site of the tolerant population was sufficient to elicit biological effects. Similarly, at a site contaminated by more than one toxicant, tolerance to some of the contaminants but not to the others would indicate that those to which tolerance was shown were the toxicants of primary concern in that ecosystem.

Table 3. Summary of effects of pH changes on fish.

pH	Effects
3.0-3.5	Toxic to most fish; some plants and invertebrates survive.
3.5-4.0	Lethal to salmonids. Roach, tench, perch, and pike survive.
4.0-4.5	Harmful to salmonids, tench, bream, roach, goldfish, and common carp; resistance increases with age. Pike can breed, but perch, bream, and roach cannot.
4.5-5.0	Harmful to salmonid eggs and fry; harmful to common carp.
5.0-6.0	Not harmful unless > 20 ppm CO_2, or high concentrations of iron hydroxides present.
6.0-6.5	Not harmful unless > 100 ppm CO_2.
6.5-9.0	Harmless to most fish.
9.0-9.5	Harmful to salmonids and perch if persistent.
9.5-10.0	Slowly lethal to salmonids.
10.0-10.5	Roach and salmonids survive short periods, but lethal if prolonged.
10.5-11.0	Lethal to salmonids; lethal to carp, tench, goldfish, and pike if prolonged.
11.0-11.5	Lethal to all fish.

Taken from European Inland Fisheries Advisory Committee (1969).

3.3 POPULATION LEVEL INDICATORS

3.3.1 Population Health Assessment

The individual effects discussed in the preceding sections provide information at the primary level of response for the population level of biological organization. Population integrators include rate processes describing reproduction and growth. Organismic level changes work through individuals to result in changes in the overall characteristics of populations (Kerr and Dickie, 1984; Wedemeyer et al., 1984; Munkittrick and Dixon, 1989a). This is the level of population health assessment, where biochemical or physiological responses are used to monitor or predict the overall health of a population (NRCC, 1985). Gentile et al. (1987) acknowledged the value of population responses since populations as the level of biological organization of concern.

Some researchers have found that population indicators are more sensitive than individual level measurements, and population growth may integrate the other parameters as the most sensitive indicator of impact. White sucker in three acid lakes (pH 4.90, 5.56 and 5.58) frequently matured at older ages and larger sizes and had shorter reproductive life spans than white sucker in two lakes of near neutral pH (6.30 and 6.35) (Trippel and Harvey, 1987a). In the acid lakes, elevated mortality rates coincided with the onset of sexual maturity whereas in more neutral lakes, mortality did not change at maturity. Ovarian weight and somatic index values were not significantly different in females from the two groups of lakes at different pH. Values of fecundity, ova dry weight, testicular weight and testicular somatic index of fish in acid lakes were either significantly greater than or equal to values in suckers from near neutral lakes (Trippel and Harvey, 1987a,b).

3.3.2 Population Dynamics

Presence or absence of populations from acidified lakes has been used to infer changes associated with man-induced degradation, and close agreement between laboratory toxicity data and field distributions over a wide area have been used for invertebrates such as *Daphnia galeata mendotae* (Keller, 1989) and fish populations (Holtze and Hutchinson, 1989; Warren-Hicks et al., 1989). Examination of processes involved in population dynamics include recruitment, age-class survival and reproductive success. The population size and age-class structure depend upon the population dynamics, and provide information on the tertiary responses of the population. The population size and age-class structure will be dependent on the population dynamics, and provide information on tertiary responses of the population. Production processes in aquatic communities are not well documented, although general patterns of succession in the macrobenthic infaunal community have been thoroughly documented.

Not many studies have examined these processes at acid mine drainage sites, despite the observations that invertebrate life history tables (Schindler, 1987) and fish population characteristics (Munkittrick and Dixon, 1989a,b) may be the most sensitive early indicators of ecosystem stress. Population size and age-class structure are dependent on population dynamics, and provide information on tertiary responses of the population.

Algal species have a variety of mechanisms which allow them to accommodate to elevated metal levels and several processes may be involved in any one population (Kuwabara and Leland, 1986). The algae, *Selenastrum capricornutum*, was demonstrated to adapt to Cu after 20 generations of Cu exposure. *Selenastrum* adapted to the same concentration when Cu was gradually increased over an 8 h period, using a specially designed apparatus that provided a transient increase in exposure concentration (Kuwabara and Leland, 1986). The most sensitive population measure of the algae's response to Cu was an increase in the duration of the lag phase, rather than exponential growth rate.

Burton et al. (1985) studied acidification effects on stream biota and organic matter decomposition in natural, low alkalinity streams using paired artificial streams. Acidification to pH 4 with sulphuric acid from approximately pH 7.2, resulted in significant decreased decomposition rates (after a period of six months) for leaf packs of white birch and sugar maple. Populations of total macroinvertebrates on leaf packs in the acid stream were substantially reduced over a 264 d period, whereas populations remained unchanged in the reference stream.

3.3.3 Bioindicators

The term "bioindicator" refers to organisms that may, by their presence or absence, be indicators of environmental "ecoregions" (Omernik, 1987; Whittier et al., 1988), pollution and/or environmental degradation (sentinel species). The bioindicator concept is also intended to include the use of organisms as monitors or accumulators of toxic substances, such as heavy metals or organic compounds. Regulating or monitoring pollution effects based on the presence or absence of an indicator organism is no longer considered to be a viable alternative by many researchers. The identification of the absence of a species does not provide any information about whether the species was originally present, the time span associated with its demise (or conditions associated with eradication) or the costs involved in remediation attempts to restore the species.

3.4 COMMUNITY/ECOSYSTEM LEVEL INDICATORS

Knowledge of the effects of low pH on aquatic ecosystems has been drawn largely from the literature on the impacts of acidification. The overall impacts of acidification, and atmospheric pollutants in general, on aquatic ecosystems have been the elimination of sensitive species, reduction in species diversity and abundance, and the reduction of organic matter (and decreased nutrients) (reviewed in Connell and Miller, 1984). In general, experimental design is difficult in the study of community and ecosystem effects, specifically because of high natural variability and lack of true replicates and controls.

3.4.1 Production Estimates

Although increased concentrations of metals and decreased pH have been suspected of lowering the production and diversity of aquatic communities, these conclusions are usually based on circumstantial correlations (reviewed in Mackie, 1987). Various techniques have been used to examine production, including photosynthesis (Nalewajko and Paul, 1985) or respiration of the microbial community (Allard and Moreau, 1985). Measurement of chlorophyll concentration, carbon assimilation (e.g. C^{14} measurements can be used), enzyme activity, oxygen evolution, pigment extraction and fluorometric estimates of chlorophyll content are all acceptable techniques that differ somewhat in the appropriateness of their application. Oxygen uptake rates of microbial communities have been shown to decline immediately after pH reduction (Allard and Moreau, 1985), as have algal production rates (Connell and Miller, 1984).

It is possible to automate measurements of oxygen, nitrogen, and phosphorus for production estimates. Lindblad et al. (1988) found that ratios such as the oxygen/nitrogen ratio, oxygen/phosphorus ratio, and perturbation index (PI; the relative change due to treatment) were more useful indicators of sublethal stress than individual measurements. These indexes are independent of biomass, and PI normalizes synchronic changes in the environment due to light, temperature, and salinity variations, which affect both treated and control systems equally.

3.4.2 Community Dynamics

Stressors modify biological communities by eliminating the most sensitive individuals and communities. This results in a void within the community which is usually filled by a more tolerant component of the ecosystem, which can result in the stressed community being more tolerant than the original one (Blanck, 1989). Community tolerance can increase by three different mechanisms: (1) the physiological adaptation of individuals, (2) selection of tolerant genotypes within a population, and (3) replacement of sensitive species by tolerant ones in a community (Blanck et al., 1988). The induction of tolerance in a community is not a remedy for toxic effects but should be considered evidence that a toxicant is a structuring factor in the community. Community disturbance is as strong an indicator of pollution-induced effects as physiological adaptation of the members of a community. The exposure level to which a community has adapted would theoretically indicate the amount of pollution-induced tolerance and, therefore, the amount of disturbance in the community. Community dynamics have been used to look at impacts of metals and acidification on a number of organizational levels.

With the realization that "(s)ingle species toxicology data... (may be) inadequate to predict population effects in ecological systems" (Taub, 1989), microcosm toxicity tests have been developed. This type of multispecies testing approach is useful because poor correlations between single-species toxicity tests and in situ toxicity tests have sometimes been noted by both environmentalist and industrialist researchers (Hellawell, 1988). Microcosm or multispecies tests fall into two major categories. The first involves testing of so-called "natural" communities; the second involves testing defined groups of organisms, so-called "standardized" aquatic microcosms (SAMs; Yount and Richter, 1986; Frithsen et al., 1989). This review will focus on natural communities, but the same principles apply to the study of communities in micro- or mesocosms.

3.4.2.1 Algal, Planktonic and Periphyton Communities

There are a number of problems associated with working with unicellular organisms (reviewed in Moore and Ramamoorthy, 1984), which include:

- an imperfect knowledge of the life history and ecology of most species,
- poor taxonomic descriptions which make species identification difficult, and
- the ability of some species to adapt to metals or form resistant spores.

Mine tailings and acid mine drainage may have several effects on plankton communities (reviewed in Parsons et al., 1986b):

- decreasing the amount of light for photosynthesis,
- potential toxicity from the accompanying metals, and
- inhibition of zooplankton feeding in the presence of fine, inorganic particles.

The actual impact(s) have been difficult to assess, and impacts are very site-specific. In controlled seawater simulated-ecosystems exposed to acid mine waste, phytoplankton blooms were delayed, zooplankton numbers and bacterial activity increased and heterotrophic zooflagellates were suppressed (Parsons et al., 1986a,b). The authors found that the end result was difficult to relate directly to the mine tailings and, at low concentrations, the tailings waste may act like any other suspended sediment.

Short-term acidification resulted in decreases in the density and diversity of a community of testate amoebae (Protozoa: Rhizopoda, Testacea) after sulphuric and nitric acid treatments (Costan and Planas, 1986). The trophic structure of *in situ* protozoan communities composed of several trophic levels (phototrophs, omnivores, bacteria, predators), were measured and analyzed for species richness, total population abundance, total phototroph abundance and number of bacterivorous species (Henebry and Ross, 1989). Zooplankton communities of small (0.04 ha) ponds exhibited a wide range of responses to a single application of Cd at 5.0 μg/L. Copepods appeared unaffected, while *Simocephalus* populations declined and algal biomass initially declined in treated ponds, recovering in 5 to 10 days (Kettle and DeNoyelles, 1986).

Blanck (1989) has suggested that periphyton can be used to indicate similar changes, and has called his indicator the Pollution-Induced Community Tolerance (PICT) (Blanck, 1989; Molander and Blank, 1989). PICT is assessed by comparing the tolerance of a metabolic process, such as photosynthesis in algal community samples, at sites representing a gradient in pollutant stress. A tolerance gradient can then be calculated by sampling different distances from an outfall source, for example, and the zone of impact determined. However, the sampling methods and organisms used to assess the community impact are not well-defined; more work is needed before a practical method is developed. The sensitivity of measuring the responses of periphyton communities has been further increased through the use of C^{14}-size fraction analysis of periphyton communities, although interpretation must be done cautiously without supporting physiological data (Rhee et al., 1989).

Diversity has proven to be a reasonable monitoring tool for the quality of algal communities. Studies on the effects of acid mine wastes on phytoplankton communities in lakes have been reported by Johnson et al. (1970). Both algal and zooplankton communities showed the maximum number of species at pH values between 6.5 and 7.0 (Connell and Miller, 1984). Thornton et al. (1987) and Payne and Ford (1988) have shown the value of phytoplankton species diversity as a tool for evaluating and monitoring acidity in lakes. Using sediment cores as an *in situ* historical record of diatom and chrysophyte distribution and abundance, they showed that acidity of lake water affects the relative distribution of these two classes of phytoplankton, diatoms and scaled chrysophytes and can be used to predict lake acidity based on cell counts (see also Dixit et al., 1988 and Dixit and Dixit, 1989).

Deniseger et al. (1986) examined a marked decrease in the number of species of periphytic algae colonizing artificial substrates, associated with waste from a mixed metal mine. Slight differences were found in spring samples while summer samples indicated major differences between sampling sites in species diversity, species evenness, and dissimilarity index. Diatom communities have been examined using a variety of indices, including biomass, number of species, similarity, diversity, equatability and relative abundance (Morris and Ross, 1989). In a study of sediment fossil diatom taxa in 28 lakes, Dixit and Dickman (1986) found that a majority of acid and alkaline indicator diatom taxa followed their assigned pH category. However, discrepancies in the pH classification of circumneutral diatoms suggested that this technique may not be easily adapted to flowing water systems. Sediment movement may cause a lack of discernible stratification and downstream shifting of diatoms concentrations (Dixit and Dickman, 1986).

Mulholland et al. (1986) examined periphyton community composition in response to stream acidification. Diatoms were the most prominent group in areas with a pH less than or equal to 5.0, while small chrysophytes and blue-green algae dominated at sites with a pH greater than or equal to 5.5. Mulholland et al. (1986) concluded that acidification could potentially reduce periphyton productivity in streams as a result of several water chemistry changes: (1) inhibition by high hydrogen ion concentrations, (2) inorganic carbon limitation resulting from reduced total inorganic carbon, (3) inhibition by elevated trace metal concentrations, particularly inorganic monomeric Al, and (4) increased phosphorus limitation resulting from adsorption and/or co-precipitation of PO_4 with Al (mobilized from watershed soils and precipitated as aluminum trihydroxide) as pH increases.

In an effort to develop multispecies toxicity tests and to improve the correlation between laboratory-based and in situ toxicity testing, Taub et al. (1989) conducted a series of experiments that culminated in an interlaboratory effort to test a defined Standard Aquatic Microcosm (SAM) protocol. The "community" consisted of ten species of algae and five species of zooplankters with well defined culture characteristics. Algal response was determined by estimating algal biovolume, i.e., total number of cells of each algal species multiplied by a predetermined mean volume per cell. Attempts to use fluorescence estimates of Chl a content were inconsistent due to slight variations in light intensities between experimental trials that influenced values of Chl a/cell (Taub, 1989). Responses of zooplankters were determined by enumeration of each species. Results showed that although the precise timing of events among experimental trials differed, the outcome and conclusions about the effects of copper exposure on the SAMs was the same. Experiments provided similar statistical differences between control and treated microcosms within the same experiment and gave the same rank order of the "day-weighted-variable," a statistic designed to characterize the "center of gravity" or mean of an unknown probability distribution of a number of variables (Taub et al., 1989).

3.4.2.2 Benthic Invertebrate Community

Benthic macroinvertebrates play a key role in stream ecosystems due to their intermediate position linking primary production and higher trophic levels (i.e., fish). Macroinvertebrate communities are accepted as a means of establishing site specific water quality criteria (Carlson et al., 1986) and have been used as ecological indicators in river systems for a number of reasons. Benthic communities show cumulative effects of present/past conditions, they have low mobility and relatively long life cycles (Wilhm, 1975), their ecological relationships are relatively well understood (Herricks and Cairns, 1982), sampling procedures are relatively well developed, the group is heterogeneous in that a single sampling technique collects a considerable number of species from a wide range of phyla, and macroinvertebrates are generally abundant (Mason, 1981).

General patterns of succession in the macrobenthic infaunal community have been well documented. Gray (1979) suggests that a particular species may dominate under severe pollution stress because of a flexible life-history, and not because of individual tolerance to adverse conditions; some other species with less flexible strategies may show increased abundance under slight pollution.

Bradt and Berg (1987) examined the macrozoobenthos of a lake in response to acidic deposition. They found that biomass, expressed as wet weight (including Odonata and Mollusca) increased in the least acidic lake (pH 4.2 -5.5), and that Ephemeroptera, Gastropoda, and Pelecypoda increased. The chironomid community showed some fluctuations in species composition, and predators increased in lakes with higher pH (mainly Tanypodinae), while more Chironomidae (including Tanypodinae and Tanytarsini) were found at the most acidic lake. At both field and experimental sites, dominant Ephemeropteran and Tanytarsinii chironomid communities were replaced by Hydropsychid caddisflies and Orthocladiini chironomids (Clements et al., 1988). Metal concentrations associated with changes at experimental sites were 12 μg/L Cu and 15-37 μg/L Zn, while field changes were associated with 105 μg/L Cu and 81 μg/L Zn (Clements et al., 1988).

Miller et al. (1989a,b) and Dixon and Munkittrick (1988) reported a decreased diversity of invertebrates and a decreased abundance, in lakes exposed to mixed metal mining waste. At AMD-contaminated sites, there was an almost complete absence of Ephemeroptera (mayflies), amphipods, Odonata (dragonflies), Plecoptera (stoneflies), Trichoptera (caddisflies) and unionid clams (Dixon and Munkittrick, 1988). Miller et al. (1989b) clearly showed that this response was dose dependent, although invertebrate body burdens of metals could not be correlated with sediment metal levels. The changes were correlated with changes at the fish population level (Munkittrick and Dixon, 1988a).

Ecological studies of the long-term effects of contaminants on benthic communities are often confounded and difficult to determine, since long term changes in species success are often indirectly related to contaminants, through long term physical and biotic changes. Interpretation can be complicated by interacting factors, including organic matter and productivity. In a study of 15 U.S. rivers with metal concentrations in excess of water quality criteria, LaPoint et al. (1984) found that it was difficult to separate the effects of metals from those of nutrient enrichment. At most sites, changes in invertebrate populations in the impact zones were characteristic of organically enriched streams, with a diverse upstream community being replaced by predominant chironomid communities and tubificids. At sites which did not show concomitant nutrient enrichment, invertebrate responses were much more variable (LaPoint et al., 1984). The benthic community changes more slowly than water chemistry (Bradt and Berg, 1987) and monitoring programs required to document community changes would have to be conducted over extended periods.

Important mechanisms for restructuring benthic communities exposed to short-term acid disturbance are physiological tolerance, behaviourial avoidance and direct interactions between populations (Hall, 1989). Under long-term stress, life cycle differences, adaptation, recolonization and direct and indirect interactions are important (Hall, 1989). The U.S. EPA has been developing a multiple community metrics approach which uses different components of structure and function of the indigenous community to describe the ecological integrity of a system (Plafkin et al., 1989). Assessment of benthic communities can be approached from a structural or functional perspective. Community structure is defined as a measurement of biotic characteristics at a specific point in time (e.g., density, diversity, and species composition). Community function is the measurement of any process (rate) of the ecosystem (e.g., species colonization rates). The biomonitoring of benthic communities is commonly done from a structural perspective because structural studies normally take less time and are more conventional, thus permitting comparisons to be easily made with benthic data from other studies (Matthews et al., 1982).

Mackie (1987) examined the tolerance of individual species of invertebrates based on their ecological role, and found that species which were shredders and scrapers were more sensitive than predators and filter feeders. However, he cautioned that tolerance levels may be more indicative of species sensitivity than functional feeding behaviour. The density of grazing macroinvertebrates may be lower at acidic sites. Reduced grazing intensity at highly acidic sites could be responsible for increased cell biovolume, chlorophyll a density, and the increasing importance of diatoms if these larger periphyton cells are preferentially or more efficiently grazed than the smaller sized cells (Mulholland et al., 1986).

Artificial substrates have been used to alleviate some of the difficulties of sampling natural substrates (e.g., lack of homogeneity in substrate quality). An artificial substrate is defined as any device used to mimic specific features of the aquatic environment into which it is placed. Artificial substrates are typically used to sample aquatic habitats that cannot be sampled effectively using conventional devices (e.g., grabs). While there are numerous types of artificial substrates, one commonly used to assess benthic macroinvertebrate communities is the rock filled wire basket. This method is well documented as an acceptable artificial substrate sampler for the monitoring of macroinvertebrate communities (Rabeni et al., 1985; Mason et al., 1973; Hellawell, 1978; Mason, 1981; Merritt et al., 1984). It should be noted that the purpose of the basket sampler is to monitor changes in macroinvertebrate communities over time and space, and not necessarily to reflect the true macroinvertebrate community. There are numerous advantages and disadvantages to artificial substrates.

Advantages are as follows:

- allow collection of data from locations that cannot be sampled effectively by other means
- permit standardized sampling
- reduce variability compared with other types of sampling
- require less operator skill than other methods
- convenient to use
- permit nondestructive sampling of an environment
- permit greater flexibility in sampling programs

Disadvantages are as follows:

- colonization dynamics incompletely known; comment: this is considered the most serious disadvantage. The only remedy to this is more comparison studies.
- nonrepresentative sampling under either natural or polluted conditions; comment: artificial substrates are used only as a relative measure, and are not to be equated with natural substrate. Substrates should be positioned as close to natural substrate as is possible.
- artificial substrates require long exposure time to obtain a sample; comment: artificial substrates are left in for 6-8 weeks.

- loss of fauna on retrieval of samplers; comment: a 250 µm net is used to retrieve substrates to prevent loss of invertebrates.
- unforeseen losses of artificial substrates; comment: place more artificial substrates in habitats than are required by the sample design.
- inconvenient to use, logistically awkward; comment: this is site specific and must be addressed on a site by site basis.

Overall, the use of artificial substrates provides community measures, as well as a relatively high degree of control for field work. However, their application in field studies needs to take into consideration the above disadvantages. Another way to use such substrates is to establish natural communities of either protozoa (Henebry and Cairns, 1980) or macroinvertebrates (Clements et al., 1988) at uncontaminated sites, and then move the artificial substrates to contaminated areas. Clements et al. (1988) found that the number of organisms, number of taxa, and abundance of dominant macroinvertebrate groups in the experimental streams reflected values observed at natural sites within 10 days. This approach also allows the testing of organisms rarely included in standard laboratory bioassays (e.g., instars of some mayflies) due to difficulties collecting and maintaining them in the laboratory. The use of artificial substrates has also been suggested for other organisms (Cairns et al., 1986), including protozoan communities.

3.4.2.3 Fish Community

Long-term detailed investigations are usually required to obtain reliable data on the density, productivity or diversity of fish communities (Moore and Ramamoorthy, 1984). Due to the complexity of data collection and interpretation, there have been few requirements for addressing the protection of the health of fish populations during impact assessments. Interpretation of data can be further complicated by recolonization of some species of fish from refugia or tributaries, while other species avoid recolonization because of behaviourial adaptation or restricted movements (Moore and Ramamoorthy, 1984).

The index of biotic integrity (IBI) is a community-based monitoring system which incorporates a wide variety of population measures into a single number, to characterize the aquatic system (Miller et al., 1988; Plafkin et al., 1989). The IBI has been used for both invertebrate and fish communities, and is presently being developed in a bioassessment protocol by the U.S. EPA and as a biological water quality criterion by a number of Midwest U.S. states (Plafkin et al., 1989). The advantage of the system is that it incorporates results into a single number, and that it can easily be adapted to focus on local conditions. However, the presentation of results as a single number means that the ability to trace impact(s) or focus on the changing community is relatively limited outside of the localized area for which it was developed.

3.4.3 Ecosystem Function

Algal production is known to decrease outside of the pH range of 6.5 to 7.0 (Connell and Miller, 1984), but little is known about the overall function of ecosystems. The term "ecosystem function" excludes population interrelationships which are usually regarded as community ecology (Schindler, 1987). Researchers have noted the need for studies which address functional changes in benthic ecosystems (e.g. Capuzzo, 1987). The appearance of abiotic zones, elevated primary productivity, profound changes in community structure and bioaccumulation of contaminants, all point to abnormalities in energy processing, nutrient cycling and a loss of information (Table 4; reviewed in Rapport, 1989).

However, we have not yet developed knowledge of ecosystem-level effects, nor of where and when such effects can be detected (Schindler, 1987). While specific indicators of ecosystem response to stress differ, Rapport (1989) described broad similarities:

"(1) there appears to be a reduction in the efficiency with which ecosystems process energy (reflected variously as a decline in community respiration, primary production, or net landscape production);

(2) there is an increase in the horizontal flow rates of nutrients; that is terrestrial systems lose nutrients and aquatic systems accumulate them;

(3) changes in community structure appear to favour biota that are short-lived, smaller, exotic, and have high reproductive rates. Such features often characterize 'weedy' or 'pest' species;

(4) ecosystem development appears to reverse in direction, that is to 'retrogress' to resemble earlier stages in which self-regulatory functions are less developed, species diversity is reduced, etc."

Future research should examine the functional characteristics of an ecosystem that govern both impact and recovery as well as pathways for pathogen and toxicant transport to man (Capuzzo, 1987).

Table 4. Response to stress at ecosystem and landscape levels, as identified in three studies -, Decrease; +, Increase; ?, not specified in source study; *, symptom present.

System Property	Ecosystem (Odum, 1985)	Ecosystem (Rapport et al., 1985)	Landscape (Godron & Forman, 1983)
Energetics			
Community respiration	-	?	?
Primary production	?	-	?
Net landscape production	?	?	-
Nutrient flow			
Horizontal transport	+	+	+
Community structure			
Species diversity	-	-	-
r-selected species	+	+	+
Short-lived species	+	+	?
Smaller biota	+	+	?
Food chain length	-	?	?
Exotic species	?	+	?
System features			
Openness	+	+	+
Succession reversal	*	*	?
Metastability	?	?	-
Negative interaction	+	?	?
Disease incidence	?	+	?
Mutualism	-	?	?
Population self-regulation	?	-	?
Resource use efficiency	-	?	?
Boundary distinctiveness	?	?	+
Boundary linearity	?	?	+

From Rapport, 1989 (references found in Rapport, 1989).

4.0 RECOMMENDED TESTING PROCEDURES

There are several problems associated with using a single assay or bioassessment technique for screening samples or monitoring toxicant/contaminant levels. Even within specific tests, the use of different species or end points can markedly alter interpretation of the resulting data. The use of a single bioassay as a screening test increases the chance that a sample will be identified as non-toxic, when it is actually toxic. The main driving force behind the use of a battery of tests is the inconsistency of results obtained when samples are evaluated in inter-test comparisons (reviewed in Munkittrick and Power, 1989). However, the main disadvantage of the battery approach is that several tests must be conducted; this can be expensive and time consuming and possibly inappropriate for screening large numbers of samples. If prior knowledge of an area is available, it should be possible to choose one or two assays based on the specific test sensitivities relative to expected sample characteristics. Caution must be used to interpret results since any summary of relative sensitivity will vary with the identity and number of compounds and organisms tested. Since it is known that metals will be the contaminants of concern in the case of acid mine drainage, tests known to be sensitive to metals should be utilized.

A battery approach to toxicity testing is required to thoroughly evaluate the toxicity of an effluent, compound or sediment. The measurements of several end points results in a more complete understanding of exposure routes, impacts and community consequences. Ideally, a battery of bioassays would completely characterize the status of the ecosystem. For comparison, screening tests can be used to identify samples or locations which require further testing. A listing of the parameters for possible inclusion in a test battery has been provided in Table 5; these tests have been used for describing the impacts of chemicals, including specific examples where effects of heavy metals and/or acidic conditions were measured. The problem becomes identifying which tests are the most valuable to use in any battery of tests.

Also, it is important that any testing procedure take into account specific modifying factors for the site which is being assessed. For example, the amount of particulate organic matter in a model ecosystem may significantly alter the effects of identical loading of the same toxicant in terms of bioavailability, accumulation of the toxicant by organisms and the productivity of the ecosystem. An effluent entering a productive stream or lake may pose little hazard, but the same material may have a very deleterious effect on a more oligotrophic system (McCarthy and Barnell, 1988). Other abiotic modifying factors which can affect metal toxicity include hardness, alkalinity and pH (Spear and Pierce, 1979; Winner, 1984; Sprague, 1985).

Table 5. Listing of parameters used for describing the impacts of chemicals with specific examples where effects of heavy metals and/or acidic conditions were measured.

Response Level	Description	Parameters	Specific Examples (where applicable)
Partitioning	Bioaccumulation	Complexation and storage Tissue burden and distribution Uptake kinetics Metabolism and excretion Bioconcentration	Metallothionein production
Neuroendocrine	Biochemical	Neuroamine and catecholamine responses	Cortisol release
Physiological	Primary Metabolic Impact	Enzyme activities	Hepatic mixed function oxidase induction, ALAD, ATPase, bacterial dehydrogenase enzymes, Microtox
		Respiration Photosynthesis Growth Excretion	opercular movement RNA/DNA, glycine incorporation in scales
	Primary Metabolic Responses	Metabolic rate Hematology	*Adenylate energy charge* Hematocrit, leucocrit, hemoglobin, red blood cells, white blood cells
		Pigmentation Osmoregulation/Ionoregulation Hormonal changes Cytological changes	Ion concentration Gill lamellar changes, lysosomal integrity
Integrators	Morphology	Various	Skeletal anomalies, tissue somatic indices

Table 5. Continued.

Response Level	Description	Parameters	Specific Examples (where applicable)
	Behavior	Sensory capacity	
		Rhythmic activities	Ventilatory/cough response
		Motor activity	
		Learning/motivation	Feeding behaviour
		Avoidance/attraction	Preference/avoidance
		Reproductive behavior	
	Growth	Feeding rate/nutrition	
		Scope for growth	
		Net growth efficiency	
		Body/organ weights	Liver and spleen changes
		Developmental rate/stages	Changes in sexual maturation
	Histopathology	Abnormal growths	Neoplasms/tumors
		Abnormal histological changes	
	Genetic	Chromosomal damage	Sister chromatid exchange
		Mutagenic/teratogenic effects	Ames testing
		Genotoxicity	SOS-chromotest
	Reproduction	Sexual maturation	
		Gamete viability/fertility	
		Larval development	
		Brood size/fecundity	
		Frequency of reproduction	
	Survival	Mortality	Lethal bioassays
		Growth, reproduction, etc.	Chronic bioassays
		Survival	Acclimation/adaptation/hormesis

Table 5. Continued.

Response Level	Description	Parameters	Specific Examples (where applicable)
Dynamics	Behaviour	Recolonization/migration	
		Aggression/predation	
		Vulnerability	
		Mating	
	Integrators	Age-class survival	
		Reproductive success	
		Density	
		Biomass	
		Diversity	
		Species richness	
		Succession	
		Nutrient cycling	
		Energy flow	
		Production	

4.1 PHYSIOLOGICAL TESTS

There are very few studies which provide any insight into the relevance of changes in the physiological performance of invertebrates, the techniques are difficult to adapt to field situations, sample sizes are extremely small and sampling can be costly and time consuming. It is also very difficult to justify regulating remediation or monitoring programs based on the physiological changes evident in invertebrate species. The recommendation regarding physiological testing will be restricted to fish in most cases.

4.1.1 Bioaccumulation

In an attempt to avoid the problems associated with interpreting muscle metal burdens in fish, there have been recent attempts to use bivalves to monitor pollution impacts over a short time period. There are several advantages to this approach, including the fact that some organic contaminants can be biomagnified to concentrations several orders of magnitude higher than they are present in the water column. This allows the detection of some contaminants in flesh samples which could not be detectable through sediment or water quality analyses. Unionid clams are known to be absent from some metal contaminated sites (Munkittrick et al., 1989b), and have some potential for monitoring metal pollution as a bioindicator and as a sentinel for bioaccumulation effects. Clams have been used as an integrator of contaminant exposure for organic contaminants and inorganic contaminants using measurements on both indigenous and transplanted clams (Tessier and Campbell, 1989; Pellerin-Massicotte et al., 1989).

Estimates of fish muscle levels are less useful for monitoring than many other tissues. There is little doubt that, at least for essential metals, the muscle compartment can be isolated from environmental elevations of metals. Bone allows a much more conservative estimate of chronic exposure, and levels parallel environmental patterns very well (Bendell-Young et el., 1986; Miller et al., 1989b). Estimates of gill contamination are also useful for looking at recent impacts, and liver and kidney burdens can provide insight into chronic levels (Dixon and Munkittrick, 1988). It is worth emphasizing, again, that metal burdens in tissue are an effect or phenomenon, and not a response. There is no established relationship between specific chemical concentrations and biological effects for most constituents of acid mine drainage. In papers containing both bioaccumulation data and biological effects, the variation in tissue concentrations associated with effects are generally extremely large.

Recommendation: **use bone metal levels of fish to indicate chronic exposure levels and gill, liver or kidney levels to illustrate recent, metabolic activity. The use of invertebrate (clam) species for monitoring requires further evaluation.**

4.1.2 Osmo/Ionoregulation

Whole body changes in ion levels are difficult to interpret without a considerable data base, and are difficult to relate to whole organism effects without carefully controlled experiments. A better estimate may be obtained through the examination of changes at the haematological level. Blood ion levels have been successfully used to document the impacts of acid and metal exposures, and the levels of Na^+ ions have been related to both survival and performance. Attempts to compare other parameters with whole organism responses have not been as successful.

Recommendation: **use the Na^+ loss bioassay to indicate potential problems during caged fish bioassays. Other blood parameters (K^+ and Cl^-) can be useful if combined with other indications of effects and relevance to whole organism responses.**

4.1.3 Cardiovascular/Respiratory

Fish cardiovascular physiological responses would appear to have limited use as a field indicator of concentrations of chemicals. Although impacts commonly show good correlation with whole organism responses, the levels of exposure required to produce easily detectable changes are relatively high. Furthermore, the impacts of additional stressors and the activity of cardiostimulants and depressors have not been widely studied. Haematological indicators may have more promise, but little is known about the correlation of red or white blood cell changes with whole organism responses.

Recommendation: **use haematological parameters if there is *a priori* evidence that impacts on blood constituents are expected. Other measurements of cardiovascular or respiratory changes can not be correlated, at this time, to levels below those soliciting changes in other, more easily measured parameters. They are not recommended for wide-spread use in field situations.**

4.1.4 Enzyme/Protein Changes

Effects of metal exposure on physiological processes are complex, variable and tend to be unpredictable, with some metals being stimulatory and some concentrations and inhibitory at others (Connell and Miller, 1984). None of the protein or enzyme changes studied have satisfied the requirements for data being available describing a) responses to metals, and b) direct relevance to metals, and c) correlated impacts at the level of the whole organism. A summary of the level of information available and relevance of various physiological indicators is provided in Table 6.

A recent review (NRCC, 1985) recommended that suitable diagnostic methods be:

- "... specific to one contaminant or one group of contaminants (and therefore useful in establishing cause-effect relationships),
- sensitive to low exposure levels (and therefore useful for providing early warning of stress), and
- responsive in a short time frame (and therefore allowing the potential for corrective action prior to population crises)."

The only parameters which fulfil these requirements at this time, are metallothionein levels, ALAD activity and ATPase activity (Table 6). Despite these conclusions, there are still enough data gaps and disadvantages with field application of these techniques that it becomes difficult to recommend widespread, indiscriminant use of any of these parameters. They should only be used to provide insight into mode of action of chemicals, and they will not respond to all components of acid mine drainage.

Although serum enzymes offer many advantages, including the processing of small sample sizes, rapid determination and non-lethal sampling of the fish (d'Appolonia and Anderson, 1980), the sampling requires strict standardization, a full knowledge of the impacts of biotic and abiotic modifying factors, and an understanding of the applicability of available assay kits. Furthermore, there has been very little evidence in the literature documenting a correlation of changes in the activity or levels of enzymes to changes at higher levels of organization. The problem with inconsistent results and high variability is a persistent one.

Table 6. Level of study and relevance of various physiological indicators.

	Studies with metals/acid	Relevance Metals/acid	Relevance to whole organism
DIRECT			
ALAD	+++	+++	+/-
AChE	+	+	+/-
MT	+++	+++	+/-
INDIRECT			
Proteins			
Corticosteroids	++	-	?
Stress Proteins	++	-	?
Neuroamines	+	-	+
In vivo Enzymes			
MFO	+	-	+
MAO	++	-	?
ATPase	+++	++	+
Misplaced Enzymes			
CAN	+	-	?
SDH	+	-	?
GPT/GOT	+	-	?

+++	Well studied.
++	Reasonbly well studied.
+	Few studies.
-	No studies were found
?	Not known.

It is unlikely that any one biochemical test can be used to document or describe fish health (NRCC, 1985). Surveys should involve multiple hypotheses so that whole organism impact, mode of action and population consequences can be evaluated. The use of biochemical methods offers promise in three areas, namely detection of states of stress, suggestion of modes of action and tentatively as tools to explain the metabolic basis for conventional measurements such as growth (Lockhart and Metner, 1984). Although dozens (or hundreds) of proteins and enzymes can be induced or turned off by exposure to chemicals, not all will be directly involved in the subsequent response of the entire organism in an understandable manner. Without a clear link describing the relevance to changes at the level of the whole organism, and isolation of the causative factors related to a biochemical change, interpretation is limited.

Recommendation: **The selection of biochemical indicators should be based upon the mechanism of toxic action. New or previously untested enzyme or protein responses must be tested thoroughly before incorporation, in terms of precision and accuracy, responsiveness to environmentally realistic exposure levels, specificity, and equipment requirements and expense (NRCC, 1985). Furthermore, tests have to be validated by field measurement, and the interactions of various biotic and abiotic modifying factors must be known. These include, for example, sex, season, reproductive state, age, size, species, nutritional state, stress level, temperature, photoperiod, water pH, hardness, dissolved oxygen and suspended solids.**

4.1.5 Biochemical Indicators of Growth

The majority of biochemical indicators of growth have been developed for use in short term laboratory exposures, as a sensitive method of detecting minor changes in growth rate. Consequently, most indicators cannot be easily adapted to field situations, where it may be more important to detect subtle long term changes. In many instances, the range of differences in biochemical indicators between sites showing subtle differences will be less than the variability introduced by modifying factors. In other cases, where the differences are more significant, subtle biochemical techniques may not be necessary for the detection of changes in growth. The exception would be the use of biochemical indicators of nutritional status to elucidate the mechanism of impact of stressors on growth.

Biochemical status indicators are dependent upon the recent physiological history of the organism, and are profoundly influenced by biological modifying factors. Measurements of RNA and adenylate energy charges are difficult to adapt to field work and require strict standardization. Techniques are further complicated by processing and equipment needs; those involving gene expression (mRNA) studies are extremely complex at present, while the utility of glycine incorporation and oxygen to nitrogen consumption ratios for field work remains uncertain.

Recommendation: **estimates of biochemical parameters of growth should be restricted to studies where an impact on growth has been demonstrated at the level of the whole organism. The techniques are extremely valuable for examining the mechanism of disruption. The biochemical indicators of nutritional status appear to be the most applicable for field work, and provide results which can be easily correlated to whole animal responses.**

4.1.6 Biochemical Indicators of Reproduction

Similar to biochemical indicators for growth, the optimal utility of these indicators is for detecting changes in reproductive effort between groups. The failure to establish clear linkages between biochemical indicators of reproduction and meaningful whole organism responses means that it is very difficult to interpret biochemical changes independent of information on the performance of the individual as a whole. Again, these techniques are better utilized as a means of understanding the relationship between an established change in reproductive effort and the contributing causative factors.

Recommendation: **estimates of biochemical parameters of reproduction should be restricted to studies where an impact on reproduction has been demonstrated at the level of the whole organism or the population. The techniques are extremely valuable for examining for the mechanism of disruption. At the present time, information linking changes in steroid levels and other biochemical parameters to changes in reproductive efficiency are not available. Studies should be designed to correlate biochemical changes with changes in reproductive efficiency.**

4.1.7 Cytological Changes

There is an inadequate understanding of the relationships between whole organism responses and cytological changes for most indicators. Furthermore, the interpretation of many histological changes is largely subjective and dependent upon the user's expertise and experience. The relevance of most histological changes to the performance of the organism as a whole is subject to speculation. An exception would be the morphology of gill tissue where changes in gill structure can be related to compensatory changes in the circulatory system, and in some instances of acute toxicity, to survival. Although lysosomal stability has been linked to growth in invertebrates, no such linkages have been described for fish.

Recommendation: **measurements of gill histological changes are valuable, and have been linked to changes at the organismal level. They can be a valuable component of many studies.**

4.1.8 Genetic Changes

Except for the bacterial test detailed previously, the techniques are subjective, relatively labour intensive, and data are not available to interpret the full consequences of increases in chromosome abnormalities in terms of population or community changes.

Recommendation: **these techniques are not justifiable as a surveillance or monitoring tool at this time.**

4.2 INDIVIDUAL LEVEL

As with the physiological indicators, changes in individual characteristics have not been widely studied in invertebrates, with a few exceptions. In most cases, recommendations at this level of organization will concentrate on fish.

4.2.1 Morphological Changes

Various parameters, including skeletal anomalies, neoplasm incidence and tissue somatic indices have been shown to respond to metals and acid impacts. There have been few studies which linked these changes to population-level impacts, although most studies attempted to link the changes with alterations at the biochemical level. Deformities may respond at levels below those influencing growth and survival, but there have been no studies which have clearly linked deformities to population-level changes. Tissue somatic indices are valuable surveillance tools, and can be easily and relatively inexpensively monitored.

Recommendation: **most morphological parameters are easily recorded, and should be monitored when other fish population data is being collected. Although changes have not been clearly linked to whole organism changes, few studies have collected sufficient data to evaluate their utility.**

4.2.2 Behaviour

Behaviourial changes observed under laboratory conditions are diverse and difficult to relate to field conditions (Connell and Miller, 1984). Cause and effect relationships between behaviourial changes and other indicators of organismal status have not been established for many contaminants. Jones et al. (1987) attempted to correlate behaviourial parameters (activity, thigmotaxis, appetite and attraction to a food extract) to biochemical blood parameters (haematocrit and plasma Cl^-, Na^+, osmolality, protein, cortisol and glucose). Reaction to acid stress was evident in arctic char (*Salvelinus alpinus*) for all parameters, but a predictive relationship between behaviourial and blood parameters was not established. Behaviourial assessments are generally tedious, time consuming and expensive to perform even in the controlled environment of the laboratory. The difficulties are magnified considerably when such tests are attempted in the field and the proportion of the affected population is difficult to determine. Although field distribution studies have demonstrated preference or avoidance in field organisms (Giattina et al., 1981; Hartwell et al., 1987a,b), there are a limited number of studies that contrast field distributions of populations with laboratory avoidance behaviour and/or toxicity (Sprague et al., 1965; Saunders and Sprague, 1967; Laughlin et al., 1978).

In a review of the behaviour of salmonid fishes exposed to episodic pH depressions, Gunn (1986) found that it cannot be concluded that decreases in fish populations were due to acidic conditions. He concluded that laboratory procedures should simulate field conditions and should be used to study acute behaviourial effects, such as avoidance, or various latent responses to episodic exposure to acidic water. Few studies have critically compared laboratory and field avoidance responses in fish (Giattina et al., 1981; Hartwell et al., 1987b). Field validation of laboratory behaviourial studies is critical for environmental health assessment.

In a thorough review of the effects of metals on fish behaviour, Atchison et al. (1987) concluded that changes in certain fish behaviours, especially cough rate and avoidance reactions, could be used as sensitive indicators of sublethal exposure to metals. However, they concluded that tests involving predator avoidance, feeding behaviour, learning, social interactions and a variety of locomotory responses have been insufficiently studied to judge their utility. Atchison et al. (1987) found that the lowest observed effect concentration for behaviourial responses was usually equal to or less than that obtained by other testing measures.

4.2.3 Growth and Reproduction

Suppression of growth and reproduction has been widely reported among invertebrate and fish species exposed to relatively low metal concentrations (Connell and Miller, 1984). Invertebrates and plants tend to be more resistant than fish species, although considerable variability has been recorded (reviewed in Connell and Miller, 1984). Growth measurements are valuable for quantifying the cumulative impacts of low level stressors on fish populations. Estimates of growth rates can also be used in a coordinated approach to evaluating population and ecosystem-level responses (Munkittrick and Dixon, 1989a,b). There are a wide number of reproductive measures which should be addressed.

Recommendation: **growth and reproductive parameters are easily measured, and should be recorded during preliminary evaluations of impacts. These findings can be used to design a more complete, detailed monitoring program once the severity and location of impact has been established.**

4.2.4 Survival

It is difficult to relate changes in the survival or bioassay organisms in laboratory situations to the field. There are many documented instances where lethal conditions in the laboratory were not substantiated in the field, and *vice versa*. Chronic elevations of parameters associated with acid mine drainage can result in increased or decreased performance of native species. This becomes of increased importance when attempting to investigate the mode of action of impacts. Survival at the individual level is of importance when lethal conditions are evident at a site with acid mine drainage. Survival should only be measured once evidence is available designating a zone of impact. This evidence would be composed of clear changes in benthic communities or absence or decreased performance of fish species in the area. The most valuable evidence would come from *in situ* bioassays using caged or tagged organisms. It becomes very difficult to interpret the meaning of changes in the growth or survival of caged organisms if they are different from resident individuals.

Bioassay protocols should have the capacity for both acute and chronic testing of growth, reproduction, and development, as well as mortality. Testing should provide information on multiple species, multiple life history stages, and multiple trophic levels, and that can be generalized to other comparable conditions/species. Use of organisms in standardized tests, allows for the development of rigorous and reliable data for the monitoring and verification of AMD impacts. Responses of such organisms can be interfaced with chemical analyses, supporting literature and sublethal threshold evaluations.

Recommendation: **survival should only be measured once evidence is available designating a zone of impact. This evidence would be composed of clear changes in benthic communities, absence of species or decreased performance of fish populations in the zone of impact. The testing should be designed to provide information of cause and effect relationships or the mode of action of local conditions.**

4.3 POPULATION AND COMMUNITY LEVEL

Significant to severe modifications in benthic community structure is the most commonly recorded response to metal pollution of streams and rivers. These changes usually involve a reduction in the abundance and numbers of species and the complete absence of sensitive species (Connell and Miller, 1984). There are a large number of procedures which have been used to characterize community structure, including a number of biotic indices (Table 7). It is most important to collect and present the information in a manner which allows interpretation of the data based on any characteristics chosen. All indices require a detailed breakdown of the types of organisms present, although different indices require a different degree of identification. The debate over the use of indicator species or selection of diversity or community indices was not examined in this review. It is not possible to design an index which would apply to all situations and locations. Most indices maintain a certain amount of site- and pollution-specificity.

The requirements of an index for use include (from Extence et al., 1987):

- "... the system should be based on established methods and it should be possible to calculate results retrospectively for historical data;
- the method should be as simple as possible to use, both in the field and in the laboratory;
- non-specialists should be able to easily appreciate the meaning of any grading or index rating;

Table 7. Summary of water quality indices.

Index Name[a]	No. of Variables	Scale	Range
Planning Indices			
Prevalence Duration Intensity (PDI) Index	[b]	Increasing	0-1
National Planning Priorities Index (NPPI)	[b]	Increasing	0-1
Priority Action Index (PAI)	[b]	Increasing	0-1
Environmental Evaluation System (EES)	78[c]	Decreasing	0-1000
Canadian National Index	[b]	Increasing	0-1
Potential Pollution Index (PPI)	3	Increasing	0-1000+
Pollution Index (PI)	[b]	Increasing	0-100+
Statistical Approaches			
Composite Pollution Index (CPI)	18	Increasing	-2 to 2
Index of Partial Nutrients	5	Decreasing	0-100
Index of Total Nutrients	5	Decreasing	0-100
Principal Component Analysis	[b]	N.A.[d]	N.A.
Harkins' Index (Kendall ranking)	[b]	Increasing	0-100+
Beta Function Index	[b]	Increasing	0-1
General Water Quality Indices			
Quality Index (QI)	10	Decreasing	0-100
Water Quality Index (NSF WQI)	9	Decreasing	0-100
Implicit Index of Pollution	13	Increasing	0-15+
River Pollution Index (RPI)	8	Increasing	0-1000+
Social Accounting System	11	Decreasing	0-100
Specific-Use Water Quality Indices			
Fish and Wildlife (FAWL) Index	9	Decreasing	0-100
Public Water Supply (PWS) Index	13	Decreasing	0-100
Index for Public Water Supply	11/13	Decreasing	0-100
Index for Recreation	12	Decreasing	0-1
Index for Dual Water Uses	31	Decreasing	-100 to 100[e]
Index for Three Water Uses	14	Increasing	0-1+

Taken from Ott (1978; in Connell and Miller, 1984).

a. When the proper name for an index is unavailable, the index characteristic is listed.
b. Any number of variables can be included.
c. Water quality variables account for 14 of the 78 variables used in this system.
d. N.A. = not applicable.
e. Index can be less -100 and can become a large negative number.

- the index should utilize as much information as practically possible from the sample, since it is the whole community and not just 'key groups' which respond to variations in water quality;

- the index should be applicable to all river types, whether they be fast or slow flowing, habitat rich or habitat poor;

- it should be possible to associate values with... existing or potential river stretch uses, and thus check for compliance with targets; and

- the index should be cost effective"

Community impacts on benthic macroinvertebrates have a direct impact on fish populations. Effects on fish populations can be direct, acting on growth, reproduction and/or survival, or indirect, operating through effects on predators or food resources. Growth, reproduction and survival tend to integrate results over an extended period of time. Population data based on maturity, fecundity and year-class strength should therefore offer insight into population fluctuations over time (Munkittrick and Dixon, 1989a,b). Fish monitoring is not presently used for most environmental impact assessments for several reasons, mainly that it is difficult to accomplish cost-effectively and that the data can be difficult to interpret proactively. These disadvantages are related to the modelling approaches that are available for handling fisheries data and not to recent developments (Munkittrick and Dixon, 1989a). Most approaches, including those currently being developed for large-scale monitoring, require a large number of assumptions and a large amount of background data which are expensive to collect (Munkittrick and Dixon, 1989a).

Older fish population monitoring approaches used estimates of fish population size. Such estimates were expensive to calculate, accurate only within an order of magnitude, and required many assumptions which were not applicable to all surveillance conditions. Present-day modelling approaches are also based on the general assumption that a standard stress imposed on two different sites would result in a common response. We know that this is not the case. Variation has been seen in the responses of several populations of fish to acidification and metal exposure (reviewed in Munkittrick and Dixon, 1989b). White sucker populations exposed to similar metal concentrations showed either an increased growth rate, increased fecundity and decreased age to maturity (McFarlane and Franzin, 1978; type I response in Munkittrick and Dixon, 1989a,b) or decreased growth rate, decreased fecundity and no change in age to maturation (Munkittrick and Dixon, 1988; type V response in Munkittrick and Dixon, 1989a,b). Variation has also been seen in the responses of species and populations to acidification (reviewed in Munkittrick and Dixon, 1989b).

Inconsistencies such as these complicate attempts to develop comprehensive *a priori* sampling protocols and hypotheses for population-based ecosystem health assessment (Munkittrick and Dixon, 1989b). A sampling program designed to examine reproductive abnormalities associated with egg survival would not be the best design for examining growth impacts or survival of adults. It is very important to identify factors associated with the responses of fish populations to stress, and to provide techniques for the rapid assessment of fish populations. Such techniques are presented in Munkittrick and Dixon (1989a,b), and involve the examination of population-based data on growth, reproduction and age distribution to gain insights into long term trends and changes. The limiting factor for application of the framework is a comparable reference data base, established from long term monitoring programs, baseline (pre-impact) data or a comparable reference site.

For a fish surveillance program to be successful, it must be responsive, cost-effective, rapid, easily adjustable to individual sites, and must provide results which are biologically meaningful. The optimal "sentinel" species used in this approach would have the following characteristics (Munkittrick and Dixon, 1989a):

- intermediate life span (long enough to show responses, but not too long to obscure impacts),
- early maturation (so that the time lag before detection of effects can be minimized),
- fast growth rate and high fecundity (so that metabolic demands are relatively high),
- be integral in the food chain,
- congregate at spawning time to facilitate collection, and
- be a spring spawner; use of a spring spawner means that fish unable to survive the overwintering period would die before spawning, and the ecosystem level impacts may be detected more quickly. The post spawning period is critical to the survival of the adult and detection of impacts; use of a fall spawner would mean that the impacts of overwinter mortalities would occur after the release of eggs. This would delay the detection of impacts for some time, especially if all of the stress related impacts occurred after spawning, or if the species spawned in areas away from the direct impacts of the drainage (tributaries or upstream refugia).

Recommendations:

(1) Benthic community responses have been well described for acid mine drainage, and should be the level at which preliminary measurements are conducted.

(2) Follow-up testing should involve an assessment of the consequences of a change in benthic communities for resident fish populations.

(3) The best understanding of the interactive impacts of unknown environmental chemicals is attained through a progression of studies from the community level through to the level of biochemical responses within individuals. The disadvantages of community and population level evaluations, which include poor specificity and obscure dose-response relationships, can be satisfied by follow up testing involving the use of appropriate biochemical tests (reviewed in Munkittrick and Dixon, 1989a) when differences are apparent.

(4) Biological indicators at all levels of organization are just that -indicators- and they require, for maximum utility, supporting data from analyses of tissue and environmental levels of contaminants, information about the history of exposure of the organisms to contaminated habitats, and some understanding of the vulnerability of the species to the particular contaminants (Sindermann, 1988).

4.4 WATER MEASUREMENTS

Common water quality characteristics which are useful to measure in the case of AMD include: temperature, turbidity, chlorophyll, dissolved oxygen, nutrient concentrations, total dissolved solids, pH, sulphate, conductivity, hardness, alkalinity and metal concentrations. Large scale water monitoring programs are invaluable for the qualitative identification of potentially sensitive areas, but are limited in their ability to quantitatively assess biological effects within any particular system (Munkittrick and Dixon, 1989a). Variation in modifying factors, physical characteristics, non-toxicant stressors and species composition require that potential problem areas be evaluated on a site specific basis.

Recommendation: temperature, turbidity, chlorophyll a, dissolved oxygen, nutrient concentrations, total dissolved solids, pH, sulphate, conductivity, hardness, alkalinity and metal concentrations should be measured in any study. It is essential to attempt to relate changes in the fish and benthos to changes in the water quality parameters, even though direct relationships may not be obvious.

5.0 DISCUSSION

The most common approach to wide-scale surveillance of aquatic ecosystems is to attempt to document the distribution and compartmentalization of contaminants and use this information to predict impacts on individual organisms and species. However, specific chemical data are of little use without a concomitant knowledge of ecosystem level changes. Biological data are required to evaluate ecosystem consequences and to provide a cumulative measure of the impact of mixtures over time.

Ecosystem health can be assessed by monitoring in two ways: a "bottom-up" or a "top-down" (reductionist) approach. Both approaches have advantages and disadvantages (Hodson, 1987a). Responses linked to detoxification processes (e.g., induction of MFOs in the case of selected organic contaminants, and metallothionein induction in the case of selected heavy metals) have a good conceptual basis for efficacy, since early changes in detoxification indices can generally be expected to be quite sensitive and precede the onset of more serious pathology at cellular and tissue levels of biological organization (Payne, 1984). Sindermann (1988) recommended a bottom-up monitoring approach consisting of pathology allied with biochemistry, then augmented by chemical analyses of tissues and environmental samples, as well as by supporting field and experimental studies. The dependence of a monitoring program on biochemical responses of individual organisms would result in many positive responses. Although bottom-up monitoring offers an increased early-warning capability, dose-response sensitivity and increased potential for causal demonstration (Hodson, 1986), the alteration of biological substrates is not necessarily indicative of lasting changes at the whole organism level.

Although cellular and biochemical measurements can be used as indicators of toxicant impact, and for helping to unravel the mechanism of effect, the significance of effects is best determined at higher levels of integration (Sprague, 1976). It is often suggested that changes in simple biochemical/physiological responses may be useful for predicting the impacts of pollutants at population and community levels of biological organization. There are serious conceptual constraints to this approach. It seems likely that such simple responses can go no further than serving as early warning systems for delineating potential impact zones (Payne et al., 1986). Decisions about cause and effect require demonstration of the source and pathway of impact, and a thorough knowledge of the biological behaviour and variability within a system. As a general rule, physicochemical criteria have probably been used with the least success in the long term management and conservation of natural ecosystems (Connell and Miller, 1984). Therefore, it is not advisable to regulate solely based on physiological changes in algae, invertebrates or fish.

The top-down approach initially monitors at the level of the population or community level. Although there is a time lag associated with changes at this level of organization, the impacts have the advantages of integrating responses over a considerable period of time, and being relatively slow to reverse. Appropriate biochemical testing can reduce the time lag (NRCC, 1985; Hodson, 1987a,b), since all chemical effects on ecosystems begin with an interaction between a contaminant and a biochemical reaction within an individual (Hodson, 1986). However, it becomes important to understand the significance and relevance of the biochemical tests selected for use.

A compromise between the two approaches would be the surveillance of ecosystems at a level offering evidence of changes at both the individual and community level. Traditional biomonitoring approaches have often involved the use of community-oriented criteria related to productivity and density of algae or the species composition and diversity of benthic communities. This information is not as valuable to environmental managers as data on fish populations, since major adjustments to waste treatment systems cannot be justified if the only alterations evident are in algal or insect communities (Moore and Ramamoorthy, 1984). Benthic community indices have been used successfully to identify the zone of influence and can be used in combination with other biological measures as evidence of impact. For benthic communities, artificial substrates have been used to alleviate some of the difficulties of sampling natural substrates; overall, the use of artificial substrates provides community measures, as well as a relatively high degree of control for field work. However, their application in field studies needs to take into consideration the disadvantages reviewed in Section 3.4.2.2.

Regardless of the approach taken, most improvements in monitoring and assessment programs have been related to the development of a comprehensive, multidisciplinary approach to problem-solving. No single approach to the problem of biological effects monitoring is fully satisfactory. Some methods are more useful than others, but the greatest insights are provided by multi-disciplinary efforts.

Attempting to regulate treatment responses on the basis of the presence or absence of a fish species is not feasible. This is one of the reasons that modelling benthic communities has been so successful. Elimination of sensitive invertebrate species allows the identification of impacts, and provides response time before the elimination of important fish species. Benthic monitoring does not provide all of the answers since the changes in some sensitive invertebrate species may not result in a meaningful permanent change in energy flow through the ecosystem.

The collection of data describing the growth, reproduction and survival of fish populations is of central relevance to the concerns for the protection of environments from the effects of acid mine drainage. Subtle changes in habitat use and population dynamics can be difficult to quantify in an acceptable time frame, unless species characteristics and sampling location are well suited to surveillance purposes. There is a need to develop a direct relationship between fish population changes and drainage characteristics. Monitoring fish populations is also of central concern to the preservation of the support of the public sector for mine operations. The purpose of monitoring is to define detectable impacts on the aquatic system. Invertebrate and planktonic species are highly sensitive to impacts, but community changes can conserve the patterns of energy flow through the food chain. Fish population changes are reflective of prolonged adverse conditions, and avoid the heavy regulative costs associated with overly conservative protection. The central problem becomes how to cost-effectively monitor the system and maintain the ability to identify responses which allow conservative reactive responses.

The entire success of the top-down approach relies on the detection of impacts before they become irreversible. This means that aspects which are monitored must provide rapid, proactive insight into population impacts. Characteristics of fish populations recommended for surveillance are reviewed in Section 4.3. Monitoring of fish populations has been used successfully to provide biologically meaningful information about ecosystem health. Effects detected at this level can then be followed up with appropriate biochemical and physiological testing. It is recommended that future research focus on using the top-down approach to identify where the impact of acid mine drainage can be measured most sensitively. With this information further, more focused efforts can be made to investigate the cause and effect relationships in organisms affected by acid mine drainage.

6.0 CONCLUSIONS

1. Use of a battery or suite of biological assessment tests which assess responses at the levels of individual, population and community is strongly recommended; no one method or level of examination will provide all the required information. Biological indicators at all levels of organization are just that -indicators- and they require, for maximum utility, supporting data from analyses of tissue and environmental levels of contaminants, information about the history of exposure of the organisms to contaminated habitats, and some understanding of the vulnerability of the species to the particular contaminants.

2. The best understanding of the effects of acid mine drainage will be reached through a progression of studies from the community level through to the level of biochemical responses within individuals. The disadvantages of these methods, which include poor specificity and obscure dose-response relationships, can be satisfied by follow up testing involving the use

of appropriate biochemical tests when differences are apparent. A key factor becomes the identification of which biochemical tests are appropriate for use. Assessment of ecosystem health utilizing this top-down approach is recommended, using appropriate biochemical testing to shorten the time lag between contaminant events and our ability to identify changes.

3. The most promising and proven biological monitoring techniques, that meet the requirements specified in Section 1.1, include the following:

- benthic community responses have been well described for acid mine drainage, and should be the level at which preliminary measurements are conducted. Follow-up testing should involve an assessment of the consequences of a change in benthic communities for resident fish populations.

- growth and reproductive parameters of fish populations are easily measured, and should be recorded during preliminary evaluations of impacts. These findings can be used to design a more complete, detailed monitoring program once the degree of impact has been established. Most morphological parameters are easily recorded, and should be monitored when other fish population data is being collected.

- survival should only be measured once evidence is available designating a zone of impact. This evidence would be composed of clear changes in benthic communities, absence of species or decreased performance of fish populations within the impact zone. The testing should be designed to provide information on cause and effect relationships or the impact mechanism related to local conditions.

- other changes at the individual, biochemical level are a valuable component of many studies, and are extremely valuable for examining for the mechanism of disruption. The selection of biochemical indicators should be based upon the mechanism of toxic action.

Measurements of gill histological changes are valuable, and have been linked to changes at the organismal level. Estimates of biochemical parameters of growth should be restricted to studies where an impact on growth has been demonstrated at the level of the whole organism. Haematological parameters should only be used if there is *a priori* evidence that impacts on blood constituents are expected. Some blood assays, such as the Na^+ loss bioassay, can be used to indicate potential problems during caged fish bioassays. Other blood parameters (K^+ and Cl^-) can be useful if combined with other indications of effects and relevance to whole organism responses. Bone metal levels can be used to indicate chronic exposure levels and gill, liver or kidney levels to illustrate recent, metabolic activity.

4. It is essential to relate changes in the fish and benthos to changes in the water quality parameters. Suitable parameters are temperature, turbidity, chlorophyll a, dissolved oxygen, nutrient concentrations, pH, sulphate, conductivity, hardness and metal concentrations.

5. The future objectives of the B.C. Acid Mine Drainage Task Force are to examine the impacts of acid mine drainage and to investigate whether water quality objectives are protective. These objectives can be met by implementing a tiered testing approach which utilizes the biomonitoring techniques recommended herein.

7.0 REFERENCES

Ahokas, J.T., N.T. Karki and A. Soivio. 1976. Mixed function mono-oxygenase of fish as an indicator of pollution of aquatic environment by industrial effluent. Bull. Environ. Contam. Toxicol. 16:270-274.

Alemu, P., G.W. Forsyth and G.P. Searcy. 1977. A comparison of parameters used to assess liver damage in sheep treated with carbon tetrachloride. Can J. Comp. Med. 41:420-427.

Allard, M. and G. Moreau. 1985. Short-term effect on the metabolism of lotic benthic communities following experimental acidification. Can. J. Fish. Aquat. Sci. 42:1676-1680.

Appleberg, M. 1985. Changes in haemolymph ion concentrations of *Astacus astacus* L. and *Pacifastacus leniusculus* (Dana) after exposure to low pH and aluminum. Hydrobiologia. 121:19-25.

Atchison, G.J., M.G. Henry and M.B. Sandheinrich. 1987. Effects of metals on fish behavior: a review. Environ. Biol. Fish. 18:11-25.

Atkinson, D.W. 1977. Cellular energy metabolism and its regulation. Academic Press, New York. 293 pp.

Baatrup, E. and G. Danscher. 1987. Cytochemical demonstration of mercury deposits in trout liver and kidney following methyl mercury intoxication: Differentiation of two mercury pools by selenium. Ecotoxicol. Environ. Safety. 14:129-141.

Baatrup, E, M.G. Nielsen and G. Danscher. 1986. Histochemical demonstration of two mercury pools in trout tissues: mercury in kidney and liver after mercuric chloride exposure. Ecotoxicol. Environ. Saf. 12:267-282.

Baker, A. and R. Brooks. 1989. Terrestrial higher plants which hyperaccumulate metallic elements-a review of their distribution, ecology and phytochemistry. Biorecovery 1:81-126.

Barron, M.G. and I.R. Adelman. 1984. Nucleic acid, protein content, and growth of larval fish sublethally exposed to various toxicants. Can. J. Fish. Aquat. Sci. 41:141-150.

Barton, B.A., G.S. Weiner and C.B. Schreck. 1985. Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. Can J. Fish. Aquat. Sci. 42:710-717.

Baumann, P.C. and R.B. Gillespie. 1986. Selenium bioaccumulation in gonads of largemouth bass and bluegill from three power plant cooling reservoirs. Environ. Toxicol. Chem. 5:695-701.

Baumann, P.C. and S.J. Hamilton. 1984. Vertebral abnormalities in white crappies, *Pomoxis annularis* Rafinesque, from Lake Decatur, Illinois and an investigation of possible causes. J. Fish Biol. 25:25-33.

Bayne, B.L. 1985. Cellular and physiological measures of pollution effect. Mar. Poll. Bull. 16:127-129.

Bayne, B.L., D.R. Living, M.N. Moore and J. Widdow. 1976. A cytological and a biochemical index of stress in *Mytilus edulis*. L. Mar. Poll. Bull. 7:221-224.

Bayne, B.L., M.N. Moore, J. Widdow, D.R. Livingstone and P. Salkeld. 1979. Measurements of the responses of individuals to environmental stress and pollution. Studies with bivalve molluscs. Philos. Trans. R. Soc. London B. 286:563-581.

Beckman, B.R. and W.S. Zaugg. 1988. Copper intoxication in chinook salmon (*Oncorhynchus tshawytscha*) induced by natural springwater: effects on gill Na+, K+-ATPase, hematrocrit, and plasma glucose. Can. J. Fish. Aquat. Sci. 45:1430-1435.

Bendell-Young, L.I., H.H. Harvey and J.F. Young. 1986. Accumulation of cadmium by white suckers (*Catostomus commersoni*) in relation to fish growth and lake acidification. Can. J. Fish. Aquat. Sci. 43:806-811.

Bengtsson, B.-E. 1983. Malformations in fish as an indicator of pollution in the Gulf of Bothnia (The Baltic Sea). Presented at the 4th Annu. Meet., Soc. Environ. Toxicol. Chem., Nov. 6-9, 1983, Arlington, Virginia.

Benson, W.H. and W.J. Birge. 1986. Detection of cadmium-binding proteins in fish chronically exposed to heavy metals. Environ. Toxicol. Chem. 6:623-626.

Benson, W.H., K.N. Baer, R.A. Stackhouse and C.F. Watson. 1987. Influence of cadmium exposure on selected hematological parameters in Freshwater teleost, *Notemigonus crysoleucas*. Ecotoxicol. Environ. Safety. 13:92-96.

Berglind, R. 1986. Combined and separate effects of cadmium, lead and zinc on ALA-D activity, growth and hemoglobin content in *Daphnia magna*. Environ. Toxicol. Chem. 5:989-995.

Berrill, M., L. Rowe, L. Hollett and J. Hudson. 1987. Response of some aquatic benthic arthropods to low pH. Annls. Soc. R. Zool. Belg. 117 (suppl.1): 117-127.

Besser, J.M. and C.F. Rabeni. 1987. Bioavailability and toxicity of metals leached from lead-mine tailings to aquatic invertebrates. Environ. Toxicol. Chem. 6:879-890.

Bettiger, W.J., D.J. Spry, K.A. Cockell, C. Young Cho and J.W. Hilton. 1986. The distribution of zinc and copper in plasma, erythrocytes and erythrocyte membranes of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 87C:445-451.

Biesinger, K.E., G.M. Christensen and J.T. Flandt. 1986. Effects of metal salt mixtures on *Daphnia magna* reproduction. Ecotoxicol. Environ. Saf. 11:9-14.

Bitton, G. 1983. Bacterial and biochemical tests for assessing chemical toxicity in the aquatic environment: a review. CRC Crit. Rev. Env. Control. 13:51-67.

Bitton, G., T. Khafif, N. Chataigner, J. Bastide, C.M. Coste and Gerap. 1986. A direct INT-dehydrogenase assay (DIDHA) for assessing chemical toxicity. Toxicity Assess. 1:1-12.

Black, J.J., E.D. Evans, J.C. Harshbarger and R.F. Zeigel. 1982. Epizootic neoplasms in fishes from a lake polluted by copper mining wastes. J. Nat. Conc. Inst. 69:915-926.

Blanck, H. 1989. Pollution-induced community tolerance (PICT) in periphyton. In: Transboundary Pollution, Abstracts of 10th Annual Meeting, Oct. 28-Nov. 2, 1989, Toronto, Ontario. Soc. Environ. Toxicol. Chem. p. 95.

Blanck, H., S.Å. Wängberg and S. Molander. 1988. Pollution-induced community tolerance - a new ecotoxicological tool. pp. 219-230. In: J. Cairns, Jr., and J.R. Pratt (eds.). Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals. ASTM STP 988. American Society for Testing and Materials, Philadelphia.

Blazer, V.S., R.E. Wolke, J. Brown and C.A. Powell. 1987. Piscine macrophage aggregate parameters as health monitors: effect of age, sex, relative weight, season and site quality in largemouth bass (*Micropterus salmoides*). Aquat. Toxicol. 10:199-215.

Bouck, G.R. 1984. Physiological responses of fish: problems and progress toward use in environmental monitoring. pp. 61-71. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries. Adv. Env. Sci. Tech. 16, Wiley & Sons, Toronto.

Bradley, R.W., C. DuQuesnay and J.B. Sprague. 1985. Acclimation of rainbow trout to zinc: kinetics and mechanism of enhanced tolerance induction. J. Fish Biol. 27:367-379.

Bradt, P.T. and M.B. Berg. 1987. Macrozoobenthos of three Pennsylvania lakes: responses to acidification. Hydrobiologia. 150:63-74.

Bratton, G.R. and E. Tiffany-Castiglioni. 1989. Use of the hematofluorometer to diagnose and predict the outcome of Pb toxicity in livestock. 79 pp. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario.

Bristow, M. and D. Nielson. 1981. Remote monitoring of organic carbon in surface waters. EPA/600/4-81-001; PB 81-168 965.

Bristow, M., D. Bundy, C. Edmonds, P. Ponto, B. Frey and L. Small. 1985. Airborne laser fluorosensor survey of the Columbia and Snake rivers: simultaneous measurements of chlorophyll, dissolved organics and optical attenuation. Int. J. Remote Sensing. 6:1707-1734.

Brown, S.B., J.G. Eales, R.E. Evans and T.J. Hara. 1984. Internal, thyroidal, and carbohydrate responses of rainbow trout (*Salmo gairdneri*) to environmental acidification. Can J. Fish. Aquat. Sci. 41:36-45.

Bryan, G. and L. Hummerstone. 1973. Indicators of heavy-metal contamination in the Looe Estuary (Cornwall) with particular regard to silver and lead. J. Mar. Biol. Assn. U.K. 57:75-92.

Burton, T.M., R.M. Stanford and J.W. Allan. 1985. Acidification effects on stream biota and organic matter processing. Can. J. Fish. Aquat. Sci. 42:669-675.

Burton, G.A. and G.R. Lanza. 1985. Sediment microbial activity tests for the detection of toxicant impacts. Aquatic Toxicology and Hazard Assessment: Seventh Symposium. ASTM STP 854. R.D. Cardwell, R. Purdy and R.C. Bahner (eds.). American Society for Testing and Materials. Philadelphia. p. 214-228.

Butler, W. and Katijima. 1975. A tripartite model for chloroplast fluorescence. pp. 13-24. In: M. Avron (ed.). Proceedings of the Third International Congress on Photosynthesis. Elsevier, Amsterdam.

Cairns, J., Jr. 1983. The case for simultaneous toxicity testing at different levels of biological organization. pp. 111-127. In: W.E. Bishop, R.D. Cardwell and B. B Heidolph (eds.). Aquatic Toxicology and Hazard Assessment: Sixth Symposium. ASTM STP 802. American Society for Testing and Materials, Philadelphia.

Cairns, J., Jr. Pratt, B.R. Niederlehner and P.V. McCormick. 1986. A simple, cost-effective multispecies toxicity test using organisms with a cosmopolitan distribution. Environm. Monitor. Assess. 6:207-220.

Cairns, V.W., P.V. Hodson and J.O. Nriagu (eds.). 1984. Contaminant Effects on Fisheries, Advances Environ. Sci. Technol. 16:1-333.

Calleja, A., J.M. Baldasano and A. Mulet. 1986. Toxicity analysis of leachates from hazardous wastes via Microtox and *Daphnia magna*. Toxicity Assess. 1:73-83.

Capuzzo, J.M. 1987. Ecological and human health concerns in ocean disposal of wastes. Mar. Pollut. Bull. 18:107-109.

Carlson, A.R., H. Nelson and D. Hammermeister. 1986. Development and validation of site-specific water quality criteria for copper. Environ. Toxicol. Chem. 5:997-1012.

Chen, T.T. 1983a. Identification and characterization of estrogen-responsive gene products in the liver of rainbow trout. Can. J. Biochem. 61:802-810.

Chen, T.T. 1983b. Development of a bioassay for effects of xenobiotic compounds on the expression of genes in fish. Final report to the great Lakes Fisheries Research Board Branch, Canada Centre for Inland Waters. Contract OSE81-00140.

Clark, K.L. and B.D. LaZerte. 1985. A laboratory study of the effects of aluminum and pH on amphibian eggs and tadpoles. Can J. Fish. Aquat. Sci. 42:1544-1551.

Clements, W.H., D.S. Cherry and J. Cairns, Jr. 1988. Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results. Can. J. fish. Aquat. Sci. 45:2017-2025.

Collvin, L. 1985. Effects of copper on growth and starvation in perch, *Perca fluviatilis* L. J. Fish. Biol. 27:757-764.

Connell, D.W. and G.J. Miller. 1984. Chemistry and Ecotoxicology of Pollution. John Wiley and Sons Inc., Toronto. 444 pp.

Costan, G. and D. Planas. 1986. Effects of a short-term experimental acidification on a microinvertebrate community: Rhizopoda, Testacea. Can J. Zool. 64:1224-1230.

Cowles, T., J. Moum, R. Desidero and M. Angel. 1989. *In situ* monitoring of ocean chlorophyll via laser-induced fluorescence backscattering through an optical fiber. Appl. Opt. 28:595-600.

Cullen, J., M. Zhu, and D. Pierson. 1986. A technique to assess the harmful effects of sampling and containment from determination of primary production. Limnol. Oceanogr. 31:1364-1373.

Cunningham, G.L. and B.J. Shuter. 1986. Interaction of low pH and starvation on body weight and composition of young-of-year smallmouth bass (*Micropterus dolomieui*). Can J. Fish. Aquat. Sci. 43:869-876.

d'Apollonia, S. and P.D. Anderson. 1980. Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and sorbitol dehydrogenase from the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 37:163-169.

Dallinger, R. and H. Kautzky. 1985. The importance of contaminated food for the uptake of heavy metals by rainbow trout (*Salmo gairdneri*): a field study. Oecologia (Berlin). 67:82-89.

Daye, P.G. and E.T. Garside. 1980. Structural alterations in embryos and alevins of the Atlantic salmon, *Salmo salar* L., induced by continuous or short-term exposure to acidic levels of pH. Can J. Zool. 58:27-43.

Delorme, P.D., S.A. Kenny and F.W. Ward. 1989. Use of brain cholinesterase activities in fish for monitoring whole-animal performance in experimental and field settings. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 168.

Deniseger, J., A. Austin and W.P. Lucey. 1986. Periphyton communities in a pristine mountain stream above and below heavy metal mining operations. Freshwat. Biol. 16:209-218.

Diamond, J., D. Mackler, M. Collins and D. Gruber. 1989. The use of invertebrate species in the development of a silver criteria for the upper New River, Virginia. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 64.

Dick, P.T. and D.G. Dixon. 1985. Changes in circulating blood cell levels of rainbow trout, *Salmo gairdneri* Richardson, following acute and chronic exposure to copper. J. Fish Biol. 26:475-481.

Dickson, G.W., J.P. Giesy and L.A. Briese. 1982. The effect of chronic cadmium exposure on phosphoadenylate concentration and adenylate energy charge of gills and dorsal muscle tissue of crayfish. Environ. Toxicol. Chem. 1:147-156.

Dixit, A. and S. Dixit. 1989. Surface-sediment chrysophytes from 35 Quebec lakes and their usefulness in reconstructing lake-water pH. Can. J. Bot. 67:2071-2076.

Dixit, S., A. Dixit and R. Evans. 1988. Scaled chrysophytes (Chrysophyceae) as indicators of pH in Sudbury, Ontario, lakes. Can. J. Fish. Aquat. Sci. 45:1411-1421.

Dixit, S.S. and M.D. Dickman. 1986. Correlation of surface sediment diatoms with the present lake water pH in 28 Algoma lakes, Ontario, Canada. Hydrobiologia. 131:133-143.

Dixon, D.G. and J.W. Hilton. 1981. Influence of available dietary carbohydrate content on tolerance of waterborne copper by rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 19:509-517.

Dixon, D.G. and J.B. Sprague. 1981a. Acclimation induced changes in toxicity of arsenic and cyanide to rainbow trout. J. Fish Biol. 18:579-589.

Dixon, D.G. and J.B. Sprague. 1981b. Acclimation to copper by rainbow trout (*Salmo gairdneri*) - a modifying factor in toxicity. Can. J. Fish. Aquat. Sci. 38:880-888.

Dixon, D.G. and K.R. Munkittrick. 1988. *In situ* assessment of copper and zinc impacts on white sucker populations of the Manitouwadge chain of lakes. Ontario Ministry of the Environment, Project 193 RR/331 PL, 209 p..

Dixon, D.G., P.V. Hodson and K.L.E. Kaiser. 1987. Serum sorbitol dehydrogenase activity as an indicator of chemically induced liver damage in rainbow trout. Environ. Toxicol. Chem. 6:685-696.

Dixon D.G., C.E.A. Hill, P.V. Hodson, E.J. Kempe and K.L.E. Kaiser. 1985. Plasma leucine aminonaphthylamidase as an indicator of acute sublethal toxicant impact in rainbow trout. Environ. Toxicol. Chem. (In Press).

Dixon, D.G., C.E.A. Hill, P.V. Hodson, E.J. Kempe and K.L.E. Kaiser. 1985. Plasma leucine aminonaphthylamidase as an indicator of acute sublethal toxicant stress in rainbow trout. Environ. Toxicol. Chem. 4:789-796.

Donaldson, E.M., U.H.M. Fagerlund and J.R. McBride. 1984. Aspects of the endocrine stress response to pollutants in salmonids. pp. 213-221. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries. Adv. Env. Sci. Tech. 16, Wiley & Sons, Toronto.

Duangsawasdi, M. and J.F. Klaverkamp. 1979. Acephate and fenitrothion toxicity in rainbow trout: effects of temperature stress and investigations on the sites of action. pp. 35-51. In: L.L. Marking and R.A. Kimerle (eds.). Aquatic Toxicology. Am. Soc. Test. Mater. Spec. Tech. Publ. 667. Philadelphia, Pennsylvania.

Duncan, D.A. and J.F. Klaverkamp. 1983. Tolerance and resistance to cadmium in white suckers (*Catostomus commersoni*) previously exposed to cadmium, mercury, zinc, or selenium. Can. J. Fish. Aquat. Sci. 40:128-138.

Duret, S., J. Bonaly, A. Bariaud, A. Vannereau and J.C. Mestre. 1986. Cadmium-induced ultrastructural changes in *Euglena* cells. Environ. Res. 39:96-103.

Dutka, B.J. 1988. Proposed ranking scheme and battery of tests for evaluating hazards in Canadian waters and sediments. NWRI Contribution No. 88-80, unpubl. MS.

Dutka, B.J., K. Walsh, K.K. Kwan, A. El Shaarawi, D.L. Liu and K. Thompson. 1986. Priority site selection for degraded areas based on microbial and toxicant screening tests. Water Poll. Res. J. Canada. 21:267-282.

Elnabarawy, M.T. 1986. Short-term microbial and biochemical assays for assessing chemical toxicity. Hazardous Substances. (Nov.): 11-14.

Erickson, J., K. Pfister, M. Rahire, R. Togasaki, L. Mets and J. Rochaix. 1989. Molecular and biophysical analysis of herbicide-resistant mutants of *Chlamydomonas reinhardtii*: structure-function relationship of the photosystem II D1 polypeptide. The Plant Cell 1:361-371.

European Inland Fisheries Advisory Committee. 1969. Water quality criteria for European freshwater fish, Report on extreme pH values and inland fisheries. Water Res. 3:593.

Extence, C.A., A.J. Bates, W.J. Forbes and P.J. Barham. 1987. Biologically based water quality management. Environ. Poll. 45:221-236.

Fabacher, D.L. 1982. Hepatic microsomes from freshwater fish. II. Reduction of benzo(a) pyrene metabolism by the fish anesthetics quinaldine sulfate and tricaine. Comp. Biochem. Physiol. 37C:285-288.

Fairbrother, A. 1989. Methods used in determination of cholinesterase activity - A U.S. perspective. 130 pp. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario.

Fõrlin, L. and T. Hansson. 1982. Effects of treated municipal wastewater on the hepatic, xenobiotic, and steroid metabolism in trout. Ecotoxicol. Environ. Safety. 6:41-48.

Forsberg, A., S. Soderland, A. Frank, L. Petersson and M. Pedersen. 1988. Studies on metal content in the brown seaweed, *Fucus vesiculosus* from archipelago of Stockholm. Environ. Pollut. 49:245-263.

Foster, P. 1976. Concentrations and concentration factors of heavy metals in brown algae. Environ. Pollut. 10:45-53.

France, R.L. and P.M. Stokes. 1987. Life stage and population variation in resistance and tolerance of *Hyalella azteca* (amphipoda) to low pH. Can. J. Fish. Aquat. Sci. 44:1102-1111.

Fraser, G.A. and H.H. Harvey. 1984. Effects of environmental pH on the ionic composition of white sucker (*Catostomus commersoni*) and pumpkinseed (*Lepomis gibbosus*). Can J. Zool. 62:249-259.

Frithsen, J., D. Nacci, C. Oviatt, C. Strobel and R. Walsh. 1989. Using single-species and whole ecosystem tests to characterize the toxicity of a sewage treatment plant effluent. pp. 231-250. In: G. Suter II and M. Lewis (eds.). Aquatic Toxicology and Environmental Fate: Eleventh Volume, ASTMSTP 1007. American Society for Testing and Materials, Philadelphia, 1989.

Fromm, P.O. 1980. A review of some physiological and toxicological responses of freshwater fish to acid stress. Env. Biol. Fish. 5:79-93.

Fryer, J.N., W.H. Tam, B. Vallentine and R.E. Tikkala. 1988. Prolactin cell cytology, plasma electrolytes, and whole-body sodium efflux in acid-stressed brook trout (*Salvelinus fontinalis*). Can J. Fish. Aquat. Sci. 45:1212-1221.

Geller, G. and P. Nobel. 1987. Comparative cactus architecture and PAR interception. Am. J. Bot. 74:998-1005.

Gentile, J.H., D.J. Scott, S.M. Lussier and M.S. Redmond. 1987. The assessment of Black Rock Harbor dredged material impacts on laboratory population responses. Tech. Rept. D-87-3. Prepared for the U.S. Army Engineer Waterways Experiment Station, Vicksburg, Miss. by the U.S. Environmental Protection Agency, Narragansett, R.I. 64 pp.

Giattina, J.D., D.S. Cherry, J. Cairns, Jr. and S.R. Larrick. 1981. Comparison of laboratory and field avoidance behavior of fish in heated chlorinated water. Trans. Am. Fish. Soc. 110:526-535.

Giesy, J.P., C.S. Duke, R.D. Bingham and G.W. Dickson. 1983. Changes in phosphoadenylate concentrations and adenylate energy charge as an integrated biochemical measure of stress: the effects of cadmium on the freshwater clam *Corbicula fluminaes*. Toxicol. Environ. Chem. 2:259-295.

Giesy, J.P., J.W. Bowling & H.J. Kamia. 1980. Cadmium and zinc accumulation in freshwater crayfish. Arch. Env. Contam. Toxicol., 9:683-697.

Gill, T.S. and J.C. Pant. 1986. Chromatin condensation in the erythrocytes of fish following exposure to cadmium. Bull Environ. Contam. Toxicol. 36:199-203.

Gingerich, W.H. 1982. Hepatic toxicology of fishes. pp. 55-103. In: L.G. Weber (ed.). Aquatic Toxicology Raven Press, New York.

Goolish, E.M. and I.R. Adelman. 1983a. Effects of fish growth rate, acclimation temperature and incubation temperature on *in vitro* glycine uptake by fish scales. Comp. Biochem. Physiol. 76A:127-134.

Goolish, E.M. and I.R. Adelman. 1983b. ^{14}C-glycine uptake by fish scales: refinement of a growth index and effects of a protein-synthesis inhibitor. Trans. Am. Fish. Soc. 112:647-652.

Gray, J.S. 1979. Pollution-induced changes in populations. Phil. Trans. R. Soc. Lond. B. 286:545-561.

Gressel, J. 1985. The molecular anatomy of resistance to photosystem II herbicides. Oxford Surv. Plant & Cell Biol. 2:321-328.

Grippo, R.S. and W.A. Dunson. 1989. Sodium loss from trout: an *in situ* bioassay for evaluation of coal mine polluted streams. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 241.

Gunn, J.M. 1986. Behaviour and ecology of salmonid fishes exposed to episodic pH depressions. Environ. Biol. Fish. 17:241-252.

Gunn, J.M. and D.L.G. Noakes. 1987. Latent effects of pulse exposure to aluminum and low pH on size, ionic composition, and feeding efficiency of lake trout (*Salvelinus namaycush*) alevins. Can. J. Fish. Aquat. Sci. 44:1418-1424.

Habig, C. and R.T. Di Giulio. 1989. Biochemical characteristics of cholinesterases in aquatic organisms. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 149.

Hall, R.J. 1989. Effects of pH disturbance on benthic community patterns at different temporal and spatial scales in eastern North America. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 110.

Hamilton, S.J. and P.M. Mehrle. 1986. Metallothionein in fish: review of its importance in assessing stress from metal contaminants. Trans. Amer. Fish. Soc. 115:596-609.

Hammer, D.A. (ed.) 1989. Constructed Wetlands for Wastewater Treatment: municipal, industrial, and agricultural. Proc. First Internat. Conf. Constructed Wetlands for Wastewater treatment, Chattanooga, TN, June 13-17, 1988. 831 p.

Hara, T.J. 1982. (ed.). Chemoreception in Fishes. Elsevier, New York.

Harrison, F.L. and J.R. Lam. 1986. Copper-binding proteins in liver of bluegills exposed to increased soluble copper under field and laboratory conditions. Environ. Health Perspect. 65:125-132.

Hartwell, S.I., D.S. Cherry and J. Cairns, Jr. 1987a. Avoidance responses of schooling fathead minnows (*Pimephales promelas*) to a blend of metals during a 9-month exposure. Environ. Toxicol. Chem. 6:177-187.

Hartwell, S.I., D.S. Cherry and J. Cairns, Jr. 1987b. Field validation of avoidance of elevated metals by fathead minnows (*Pimephales promelas*) following *in situ* acclimation. Environ. Toxicol. Chem. 6:189-200.

Hartwell, S.I., D. Cherry and J. Cairns, Jr. 1988. Fish behavioral assessment of pollutants. pp. 138-165. In: J. Cairns, Jr. and J.R. Pratt (eds.). Functional testing of aquatic biota for estimating hazards of chemicals, ASTM STP 988. American Society for Testing and Materilas, Philadelphia.

Haux, C., Å. Larsson, G. Lithner and M.J. Sjöbeck. 1986. A field study of physiological effects on fish in lead-contaminated lakes. Environ. Toxicol. Chem. 5:283-288.

Hawkins, W.E., L.G. Tate, T.G. Sarphie. 1980. Acute effects of cadmium on the spot *Leiostomus zanthurus* (teleostei): tissue distribution and renal ultrastructure. J. Toxicol. Environ. Health. 6:283-295.

Heath, A.G. 1984. Changes in tissue adenylates and water content of bluegill, *Lepomis macrochirus*, exposed to copper. J. Fish Biol. 24:299-309.

Heinz, G.H. 1989. How lethal are sublethal effects? Environ. Toxicol. Chem. 8:463-464.

Hellawell, J.M. 1978. Biological Surveillance of Rivers. Water Research Centre, England. 332 pp.

Hellawell, J. 1988. Toxic substances in rivers and streams. Env. Poll. 50:61-85.

Hendricks, A.C. 1978. Response of *Selanastrum capricornutum* to zinc sulfides. J. Wat. Pollut. Control Fed. 50:163-168.

Henebry, M.S. and J. Cairns, Jr. 1980. Monitoring of stream pollution using protozoan communities on artificial substrates. Trans. Am. Micros. Soc. 99:151-160.

Henebry, M.S. and P.E. Ross. 1989. Changes in the trophic structure of protozoan communities exposed to contaminated sediments. Proc. 10th Soc. Env. Toxicol. Chem. Toronto, Ontario. p. 151.

Herricks, E.E. and J. Cairns, Jr. 1982. Biological Monitoring. Part III - Receiving system methodology based on community structure. Water Resources. 16:141-153.

Hille, S. 1982. A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri*, Rich. J. Fish Biol. 20:535-569.

Höbe, H. 1987. Sulphate entry into soft-water fish (*Salmo gairdneri, Catostomus commersoni*) during low ambient pH exposure. J. Exp. Biol. 133:87-109.

Höbe, H., P.R.H. Wilkes, R.L. Walker, C.M. Wood and B.R. McMahon. 1983. Acid-base balance, ionic status, and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*). Can. J. Zool. 61:2660-2668.

Hodson, P.V. 1986. Water quality criteria and the need for biochemical monitoring of contaminant effects on aquatic ecosystems. 140 pp. In: B.T. Hart (ed.), Water Quality Management Freshwater Ecotoxicity in Australia. Water Studies Centre, Chisholm, Inst. Technol. Melbourne, Australia.

Hodson, P.V. 1987a. Indicators of ecosystem health at the species levels and the example of selenium effects on fish. Presented at A Workshop on Ecological Indicators, Univ. of Toronto, June, 1987.

Hodson, P.V. 1987b. The effect of metal metabolism on uptake, disposition and toxicity in fish. Aquat. Toxicol. In Press.

Hodson, P.V. and B.R. Blunt. 1986. The effect of time from hatch on the yolk conversion efficiency of rainbow trout, *Salmo gairdneri*. J. Fish Biol. 29:37-46.

Hodson, P.V., J.W. Hilton, B.R. Blunt and S.J. Slinger. 1980. Effects of dietary ascorbic acid on chronic lead toxicity to young rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 34:170-176.

Hodson, P.V., B.R. Blunt and D.M. Whittle. 1984. Monitoring lead exposure of fish, pp. 87-97. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries, Advances Environ. Sci. Technol. Vol. 16.

Hoge, F. and R. Swift. 1981. Application of the NASA airborne oceanographic lidar to the mapping of chlorophyll and other organic pigments. pp. 349-374. In: Chesapeake Bay Plume Study Superflex 1980, NASA Conf. Publ. 2188 (U.S. GPO, Washington, DC, 1981).

Hoge, F. and R. Swift. 1983. Airborne dual laser excitation and mapping of phytoplankton in a Gulf Stream Warm Core Ring. Appl. Opt. 22:2272-2281.

Hoge, F. and R. Swift. 1985. Airborne mapping of laser-induced fluorescence of chlorophyll a and phycoerythin in a Gulf Stream Warm Core Ring. pp. 353-372. In: Mapping Strategies in Chemical Oceanography. A. Zirino (ed.), Advances in Chemistry series, American Chemical Society.

Hokanson, K.E.F. and L.L. Smith. 1971. Some factors influencing toxicity of linear alkylate sulfonate (LAS) to the bluegill. Trans. Am. Fish. Soc. 100:1-12.

Holdway, D.A. and D.G. Dixon. 1986. Effects of methoxychlor exposure of flagfish eggs (*Jordanella floridae*) on hatchability, juvenile methoxychlor tolerance and whole-body levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid. Water Res. 20:983-897.

Holdway, D.A., B.D. Sloley, R.G.H. Downer and D.G. Dixon. 1986. Effect of pulse exposure to methoxychlor on brain serotonin levels in American flagfish (*Jordanella floridae*, Goode and Bean) as modified by time after exposure, concentration and gender. Environ. Toxicol. Chem. 5:289-294.

Holdway, D.A., B.D. Sloley, K.R. Munkittrick, R.G.H. Downer and D.G. Dixon. 1988. Effects of gender and reproductive status on brain catecholamine and indoleamine levels of flagfish (*Jordanella floridae*). Fish. Physiol. Biochem. 5:153-158.

Hollister, T.A. and G.E. Walsh. 1973. Differential responses of marine phytoplankton to herbicides: oxygen evolution. Bull. Environ. Contam. Toxicol. 9:291-295.

Holtze, K.E. and N.J. Hutchinson. 1989. Lethality of low pH and Al to early life stages of six fish species inhabiting PreCambrian Shield waters in Ontario. Can J. Fish. Aquat. Sci. 46:1188-1202.

Ivanovici, A.M. 1980. Application of adenylate energy charge to problems of environmental impact assessment in aquatic organisms. Helgol. Wiss. Meeresunters. 33:556-565.

Jagoe, C.H. and T.A. Haines. 1985. Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Can. J. Zool. 63:130-138.

Jahan-Parwar, B. 1989. Neurobehavioral toxicology of polychlorinated biphenyls in a marine mollusc: I. neurological effects. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 83.

James, M.O. and J.R. Bend. 1980. Polycyclic aromatic hydrocarbon induction of cytochrome P-450-dependent mixed-function oxidases in marine fish. Toxicol. Appl. Pharmacol. 54:117-133.

Johnson, R.D. and H.L. Bergman. 1984. Use of histopathology in aquatic toxicology: a critique. pp. 19-36. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries. Adv. Env. Sci. Tech. 16, Wiley & Sons, Toronto.

Johnson, M., M. Michalski and A. Christie. 1970. Effects of acid mine wastes on phytoplankton communities of two Northern Ontario Lakes. J. Fish. Res. Board Can. 27:427-444.

Jones, K.A., S.B. Brown and T.J. Hara. 1987. Behavioral and biochemical studies of onset and recovery from acid stress in Arctic char (*Salvelinus alpinus*). Can. J. Fish. Aquat. Sci. 44:373-381.

Kaplan, A. and O. Bjorkman. 1980. Ratio of CO_2 (carbon dioxide) uptake to O_2 (oxygen) evolution during photosynthesis in higher plants (*Encelia californica, Atriplex rosea*). Zeitsch. Pflanenphys. 96:185-188.

Karl, D.M. 1980. Cellular nucleotide measurements and applications in microbial ecology. Microbiol. Rev. 44:739-796.

Karlsson-Norrgren, L., I. Bjorklund, O. Ljungberg and P. Runn. 1986. Acid water and aluminium exposure: experimentally induced gill lesions in brown trout, *Salmo trutta* L. J. Fish Dis. 9:11-25.

Kay, J., D.G. Thomas, M.W. Brown, A. Cryer, D. Shurben, J.F. deL. G. Solbe and J.S. Garvey. 1986. Cadmium accumulation and protein binding patterns in tissues of the rainbow trout, *Salmo gairdneri*. Environ. Health Perspect. 65:133-139.

Kearns, P.K. and G.J. Atchison. 1979. Effects of trace metals on growth of yellow perch (*Perca flavescens*) as measured by RNA-DNA ratios. Environ. Biol. Fish. 4:383-387.

Keller, W., N.D. Yan, K. Holtze and J.R. Pitblade. 1989. Effects of acidification on *Daphnia galeata mendotae*. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 106.

Kerr, S.R. and L.M. Dickie. 1984. Measuring the health of aquatic ecosystems. pp. 279-284. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.), Contaminant Effects on Fisheries, Adv. Env. Sci. Tech. 16, Wiley and Sons, Toronto.

Kettle, W.D. and F. deNoyelles, Jr. 1986. Effects of cadmium stress on the plankton communities of experimental ponds. J. Freshwat. Ecol. 3:433-443.

Khan, A.T. and J.S. Weis. 1986. Effects of methylmercury on sperm and egg viability of two populations of killifish (*Fundulus heteroclitus*). Arch. Environ. Contam. Toxicol. 16:499-505.

Kito, H., Y. Ose and T. Sato. 1986. Cadmium-binding protein (metallothionein) in carp. Environ. Health Perspect. 65:117-124.

Klaverkamp, J.F. 1982. The physiological pharmacology ad toxicology of fish cardiovascular systems. pp. 1-54. In: I.J. Weber (ed.). Aquatic toxicology. Raven Press, New York. pp. 1-54.

Klaverkamp, J.F. and B.R. Hobden. 1980. Brain acetylcholinesterase inhibition and hepatic activation of acephate and fenitrothion in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 37:1450-1453.

Klaverkamp, J.F. and D.A. Duncan. 1987. Acclimation to cadmium toxicity by white suckers: cadmium binding capacity and metal distribution in gill and liver cytosol. Environ. Toxicol. Chem. 6:275-289.

Klaverkamp, J.F., W.A. Macdonald, D.A. Duncan and R. Wagemann. 1984. Metallothionein and acclimation to heavy metals in fish: a review. pp. 99-113. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects of Fisheries. Adv. Env. Sci. Tech. 16, Wiley & Sons, Toronto.

Kleinow, K.M. and A.S. Brooks. 1986a. Selenium compounds in the fathead minnow (*Pimephales promelas*) - I. Uptake, distribution and elimination of orally administered selenate, selenite and *l*-selenomethionine. Comp. Biochem. Physiol. 83C:61-69.

Kleinow, K.M. and A.S. Brooks. 1986b. Selenium compounds in the fathead minnow (*Pimephales promelas*) - II. Quantitative approach to gastrointestinal absorption, routes of elimination and influence of dietary pretreatment. Comp. Biochem. Physiol. 83C:71-76.

Klerks, P.L. and J.S. Weis. 1987. Genetic adaptation to heavy metals in aquatic organisms: a review. Env. Pollut. 45:173-205.

Klontz, G.W. 1984. Epidemiology of diseases in wild fish populations, pp. 9-18. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries, Adv. Env. Sci. Tech. 16, Wiley & Sons, Toronto.

Knowles, C.O. and M.J. McKee. 1987. Protein and nucleic acid content in *Daphnia magna* during chronic exposure to cadmium. Ecotoxicol. Environ. Safety. 13:290-300.

Krantzberg, G. 1989. Accumulation of essential and nonessential metals by chironomid larvae in relation to physical and chemical properties of the elements. Can. J. Fish. Aquat. Sci. 46:1755-1761.

Krantzberg, G. and P.M. Stokes. 1989. Metal regulation, tolerance and body burdens in the larvae of the genus *Chironomus*. Can. J. Fish. Aquatic Sci., 46:389-398.

Kurelic, B.Z., M. Matjasevic, M. Rijavic, M. Alacevic, S. Britvic, W.E.G. Müller and R.K. Zahn. 1979. Induction of benzo(a) pyrene monooxygenase in fish and the *Salmonella* test as a tool for detecting mutagenic-carcinogenic xenobiotics in the aquatic environment. Bull. Environ. Contam. Toxicol. 21:799-807.

Kuwabara, J.S. and H.V. Leland. 1986. Adapation of *Selanastrum capricornutum* (chlorophyceae) to copper. Environ. Toxicol. Chem. 5:197-203.

Lacroix, G.L. 1985. Plasma ionic composition of the Atlantic salmon (*Salmo salar*), white sucker (*Catostomus commersoni*), and alewife (*Alosa pseudoharengus*) in some acidic rivers of Nova Scotia. Can. J. Zool. 63:2254-2261.

Lacroix, G.L., D.J. Gordon and D.J. Johnston. 1985. Effects of low environmental pH on the survival, growth, and ionic composition of postemergent Atlantic salmon (*Salmo salar*). Can J. Fish. Aquat. Sci. 42:768-775.

Landner, L., A.H. Neilson, L. Sörensen, A. Tärnholm and T. Viktor. 1985. Short-term test for predicting the potential of xenobiotics to impair reproductive success in fish. Ecotoxicol. Environ. Saf. 9:282-293.

Lanno, R.P. and D.G. Dixon. 1989. Physiological response of juvenile rainbow trout to chronic waterborne thiocyanate exposure. Proc. 10th Soc. Env. Toxicol. Chem. Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 260.

Lanno, R.P., B. Hicks and J.W. Hilton. 1987. Histological observations on intrahepatocytic copper-containing granules in rainbow trout reared on diets containing elevated levels of copper. Aquat. Toxicol. 10:251-263.

LaPoint, T.W., S.M. Melancon and M.K. Morris. 1984. Relationships among observed metal concentrations, criteria, and benthic community structural responses in 15 streams. J. Water Pollut. Control Fed. 56:1030-1038.

Larsson, Å., C. Haux and M.-L. Sjöbeck. 1985. Fish physiology and metal pollution: results and experiences from laboratory and field studies. Ecotoxicol. Environ. Saf. 9:250-281.

Laughlin, R.A. C.R. Cripe and R.J. Livingston. 1978. Field and laboratory avoidance reactions by blue crabs (*Callinectes sapidus*) to storm water run-off. Trans. Am. Fish. Soc. 107:78-86.

Laurén, D.J. and D.G. McDonald. 1986. Influence of water hardness, pH, and alkalinity on the mechanisms of copper toxicity in juvenile rainbow trout, *Salmo gairdneri*. Can J. Fish. Aquat. Sci. 43:1488-1496.

Laurén, D.J. and D.G. McDonald. 1987a. Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. Can J. Fish. Aquat. Sci. 44:99-104.

Laurén, D.J. and D.G. McDonald. 1987b. Acclimation to copper by rainbow trout, *Salmo gairdneri*: biochemistry. Can J. Fish. Aquat. Sci. 44:105-111.

Lech, J.J., M.J. Vodicnik and C.R. Elcombe. 1982. Induction of monooxygenase activity in fish. Aquatic Toxicology. Raven Press, New York. pp. 107-140.

Lee, R.F. 1981. Mixed-function oxygenases (MFO) in marine invertebrates. Mar. Biol. Lett. 2:87-105.

Leino, R.L. and J.H. McCormick. 1984. Morphological and morphometrical changes in chloride cells of the gills of *Pimephales promelas* after chronic exposure to acid water. Cell Tissue. Res. 236:121-128.

Leino, R.L., P. Wilkinson and J.G. Anderson. 1987. Histopathological changes in the gills of pearl dace, *Semotilus margarita*, and fathead minnows, *Pimephales promleas*, from experimentally acidified Canadian lakes. Can J. Fish. Aquat. Sci. 44:126-134.

Lemly, A.D. and R.J.F. Smith. 1985. Effects of acute exposure to acidified water on the behaviourial response of fathead minnows, *Pimephales promelas*, to chemical feeding stimuli. Aquat. Toxicol. 6:25-36.

Lemly, A.D. and R.J.F. Smith. 1987. Effects of chronic exposure to acidified water on chemoreception of feeding stimuli in fathead minnows (*Pimephales promelas*): mechanisms and ecological implications. Environ. Toxicol. Chem. 6:225-238.

Lett, P.F., G.J. Farmer and F.W.H. Beamish. 1976. Effect of copper on some aspects of the bioenergetics of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can. 33:1335-1342.

Lindblad, C., U. Kautsky and N. Kautsky. 1988. An *in situ* system for evaluating effects of toxicants on the metabolism of littoral communities. pp. 97-105. In: J. Cairns, Jr. and J.R. Pratt (eds.). Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals. ASTM STP 988, American Society for Testing and Materials, Philadelphia.

Little, E.E., A.J. DeLonay, R.D. Archeski and L. Cleveland. 1989. Comparative behavioral effects of surface water acidification on salmonids. Proc. 10th Soc. Env. Toxicol. Chem. Toronto, Ontario. p. 244.

Liu, D. 1985. Effect of bacterial cultures on microbial toxicity assessment. Bull. Environ. Contam. Toxicol. 34:331-339.

Liu, D. 1986. Resazurin reduction method for toxicity assessment of water soluble and insoluble chemicals. Toxicity Assess. 1:253-258.

Lockhart, W.L. and D.A. Metner. 1984. Fish serum chemistry as a pathology tool. pp. 73-85. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant effects on fisheries. John Wiley and Sons, Toronto, Ontario.

Louma, S.N. 1977. Detection of trace contaminant effects in aquatic ecosystems. J. Fish. Res. Board Can. 34:436-439.

Lowe-Jinde, L. and A.J. Niimi. 1986. Hematological characteristics of rainbow trout, *Salmo gairdneri* (Richardson), in response to cadmium exposure. Bull. Environ. Contam. Toxicol. 37:375-381.

Luxon, P.L., P.V. Hodson and U. Borgmann. 1987. Hepatic aryl hydrocarbon hydroxylase activity of lake trout (*Salvelinus namaycush*) as an indicator of organic pollution. Environ. Toxicol. Chem. 6:649-657.

Mackie, G.L. 1987. Effects of acidifying environments on freshwater mollusks in southern Ontario, Canada. Amer. Malacol. Bull. 5:31-39.

Mackie, G.L. 1989. Tolerances of five benthic invertebrates to hydrogen ions and metals (Cd, Pb, Al). Arch. Environ. Contam. Toxicol. 18:215-223.

Malins, D.C., B.B. McCain, D.W. Brown, S.L. Chan, M.S. Myers, J.T. Landahl, P.G. Prohaska, A.J. Friedman, L.D. Rhodes, D.G. Burrows, W.D. Gronlund and H.O. Hodgins. 1984. Chemical pollutants in sediments and diseases of bottom-dwelling fish in Puget Sound, Washington. Environ. Sci. Technol. 18:705-713.

Malins, D.C., M.M. Krahn, M.S. Myers, L.D. Rhodes, D.W. Brown, C.A. Krone, B.B. McCain and S.L. Chan. 1985. Toxic chemicals in sediments and biota from a creosote-polluted harbor: relationships with hepatic neoplasms and other hepatic lesions in English sole (*Parophrys vetulus*). Carcinogenesis. 6:1463-1469.

Malins, D.C., B.B. McCain, M.S. Myers, D.W. Brown, M.M. Krahn, W.T. Roubal, M.H. Schiewe, J.T. Landahl and S.L. Chan. 1988. Field and laboratory studies of the etiology of liver neoplasms in marine fish from Puget Sound. Environ. Health Pers. 71:5-16.

Malte, H. 1986. Effects of aluminum in hard, acid water on metabolic rate, blood gas tensions and ionic status in the rainbow trout. J. Fish Biol. 29:187-198.

Mason, C.F. 1981. Biology of Freshwater Pollution. Longman Inc., New York. 250 pp.

Mason, W.T., C.I. Weber, P.A. Lewis and E.C. Julian. 1973. Factors affecting the performance of basket and multiplate macroinvertebrate samplers. Freshw. Biol. 3:409-436.

Mattews, R.A., A.L. Buikema Jr., J. Cairns Jr. and J.H. Rogers Jr. 1982. Biological monitoring: Part IIA. Receiving system functional methods, relationships and indices. Water Resources. 16:129-139.

Mauro, N.A. and G.W. Moore. 1987. Effects of environmental pH on ammonia excretion, blood pH, and oxygen uptake in fresh water crustaceans. Comp. Biochem. Physiol. 87C:1-3.

McCarter, J.A. and M. Roch. 1984. Chronic exposure of coho salmon to sublethal concentrations of copper. III. Kinetics of metabolism of metallothionein. Comp. Biochem. Physiol. 77C:83-87.

McCarthy, J.F. and S.M. Barnell. 1988. How the trophic status of a community can alter the bioavailability and toxic effects of contaminants. pp. 3-16. In: J. Cairns, Jr. and J.R. Pratt (eds.), Functional testing of Aquatic Biota for Estimating Hazards of Chemicals. ASTM STP 988. American Society for Testing and Materials, Philadelphia, 1988.

McDonald, D.G. 1983. The effects of H^+ upon the gills of freshwater fish. Can. J. Zool. 61:691-703.

McFarlane, G.A. and W.G. Franzin. 1978. Elevated heavy metals: a stress on a population of which suckers *Catostomus commersoni*, in Hammell Lake, Saskatchewan. J. Fish. Res. Board Can. 35:963-970.

McKee, M.J. 1989. Total RNA content as a biomarker of toxicant effects for invertebrates: laboratory and field applications. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 156.

McKee, M.J., A.C. Hendricks and R.E. Ebel. 1983. Effects of naphthalene on benzo(a)pyrene hydroxylase and cytochrome P-450 in *Fundulus heteroclitus*. Aquat. Toxicol. 3:103-114.

McKeown, B.A., G.H. Geen, T.A. Watson, J.F. Powell and D.B. Parker. 1985. The effect of pH on plasma electrolytes, carbonic anhydrase and ATPase activities in rainbow trout (*Salmo gairdneri*) and largescale suckers (*Catostomus macrocheilus*). Comp. Biochem. Physiol. 80A:507-514.

Mehrle, P.M., T.A. Haines, S. Hamilton, J.L. Ludke, F.L. Mayer and M.A. Ribick. 1982. Relationship between body contaminants and bone development in east-coast striped bass. Trans. Am. Fish. Soc. 111:231-241.

Meisner, J.D. and W.Q. Hum. 1987. Acute toxicity of zinc to juvenile and subadult rainbow trout, *Salmo gairdneri*. Bull. Environ. Contam. Toxicol. 39:898-902.

Merritt, R.W., K.W. Cummins, V.H. Resh. 1984. Collecting, sampling, and rearing methods for aquatic insects. pp. 11-26. In: R.W. Merritt and K.W. Cummins (eds.). An Introduction to Aquatic Insects of North America. Kendall/Hunt Publishers. Iowa.

Michibata, H., S. Sahara and M.K. Kojima. 1986. Effects of calcium and magnesium ions on the toxicity of cadmium to the egg of the teleost, *Oryzias latipes*. Environ. Res. 40:110-114.

Michibata, H., Y. Nojima and M.K. Kojima. 1987. Stage sensitivity of eggs of the Teleost *Oryzias latipes* to cadmium exposure. Environ. Res. 42:321-327.

Miller, D., P. Leonard, R. Hughes, J. Karr, P. Moyle, L. Schrader, B. Thompson, R. Daniels, K. Fausch, G. Fitzhugh, J. Gammon, D. Halliwell, P. Angermeier and D. Orth. 1988. Regional applications of an index of biotic intergrity for use in water resource management. Fisheries 13:12-20.

Miller, P.A., C. Milligan and D.G. Dixon. 1989a. Relative contributions of dietary *vs.* waterborne copper and zinc to the tissue metal burdens in feral white sucker. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 264.

Miller, P.A., K.R. Munkittrick, D.G. Dixon and D.R. Barton. 1989b. Impacts of copper and zinc contamination on macroinvertebrate distribution and tissue metals levels in white sucker (*Catostomus commersoni*). Submitted to Water Air Soil Pollut.

Milner, N.J. 1979. Zinc concentrations in juvenile flatfish. J. Mar. Biol. Ass. U.K., 59:761-75.

Mitz, S.V. and J.P. Giesy. 1985. Sewage effluent biomonitoring. Ecotoxicol. Environ. Saf. 10:22-39.

Mizrahi, L. and Y. Achituv. 1989. Effect of heavy metals ions on enzyme activity in the Mediterranean Mussel, *Donax truculus*. Bull Environ. Contam. Toxicol. 42:854-859.

Mohr, L.C. and S.M. Chalanchuk. 1985. The effect of pH on sperm motility of white suckers, *Catostomus commersoni*, in the experimental lakes area. Env. Biol. Fish. 14:309-314.

Moore, J.W. and S. Ramamoorthy. 1984. Heavy Metals in Natural Waters. Springer-Verlag. New York. 268 pp.

Molander S. and H. Blanck. 1989. Pollution-induced community tolerance (pict) in periphyton established under diuron (DCMU) stress. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 199.

Moore, M.N. 1980. Cytochemical determination of cellular responses to environmental stressors in marine organisms. pp. 7-15. In: A.D. McIntyre and J.B. Pearce, (eds.). Biological effects of marine pollution and the problems of monitoring. Rapp. P.-V. Reun. Cons. Int. Explor. Mer. Vol. 179.

Moore, M.N. 1985. Cellular responses to pollutants. Mar. Pollut. Bull. 16:134-139.

Moore, M.N. and A.R.D. Stebbling. 1976. The quantitative cytochemical effects of three metal ions on a lysosomal hydrolase of a hydroid. J. Mar. Biol. Assoc. U.K. 56:995-1005.

Morris, T.L. and P.E. Ross. 1989. Using periphyton communities in ecotoxicological assessments: A case study. Proc. 10th Soc. Env. Toxicol. Chem. Toronto, Ontario. pp. 260.

Mount, D.R., J.R. Hockett and W.A. Gern. 1988a. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 2. Vitellogensis and osmoregulation. Can. J. Fish. Aquat. Sci. 45:1633-1642.

Mount, D.R., C.G. Ingersoll, D.D. Gulley, J.D. Fernandez, T.W. LaPoint and H.L. Bergman. 1988b. Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity and progeny survival. Can. J. Fish. Aquat. Sci. 45:1623-1632.

Mulholland, P.J., J.W. Elwood, A.V. Palumbo and R.J. Stevenson. 1986. Effect of stream acidification on periphyton composition, chlorophyll, and productivity. Can. J. Fish. Aquat. Sci. 43:1846-1858.

Munkittrick, K.R. and D.G. Dixon. 1988a. Growth, fecundity and energy stores of white sucker (*Catostomus commersoni*) exposed to elevations of copper and zinc. Can. J. Fish. Aquatic Sci., 45:1355-1365.

Munkittrick, K.R. and D.G. Dixon. 1988b. Evidence for a maternal yolk factor associated with increased tolerance and resistance of feral white sucker (*Catostomus commersoni*) to waterborne copper. Ecotox. Environm. Safety 15:7-20.

Munkittrick, K.R. and D.G. Dixon. 1989a. Use of white sucker (*Catostomus commersoni*) populations to assess the health of aquatic ecosystems exposed to low-level contaminant stress. Can. J. Fish. Aquat. Sci. 46:1455-1462.

Munkittrick, K.R. and D.G. Dixon. 1989b. An holistic approach to ecosystem health assessment using fish population characteristics. Hydrobiolgia: In Press.

Munkittrick, K.R. and D.G. Dixon. 1989c. The effects of natural exposure to copper and zinc on egg size and larval copper tolerance in white sucker. Ecotox. Environ. Safety. In Press.

Munkittrick, K.R. and E.A. Power. 1989. An evaluation of the sensitivity of microassays relative to trout and daphnid acute lethality studies. Unpublished Report No. EE-17, Environmental Protection Directorate, Environment Canada, Ottawa, Canada. 55 p. + appendices

Munkittrick, K.R., R.J. Martin and D.G. Dixon. 1989. Seasonal changes in whole brain amine levels in white sucker exposed to elevated levels of copper and zinc. Can. J. Zool. In Press.

Murphy, B.R., G.J. Atchison, A.W. McIntosh & D.J. Kolar. 1978. Cadmium and zinc content of fish from an industrially contaminated lake. J. Fish Biol. 13:327-335.

Nalewajko, C. and B. Paul. 1985. Effects of manipulations of aluminum concentrations and pH on phosphate uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 42:1946-1953.

Neale, P. and P. Richerson. 1987. Photoinhibition and the diurnal variation of phytoplankton photosynthesis. I. Development of a photosynthesis-irradiance model from studies of *in situ* responses. J. Plank. Res. 9:167-193.

Nebeker, A.V., A. Stinchfield, C. Savonen and G.A. Chapman. 1986a. Effects of copper, nickel and zinc on three species of oregon freshwater snails. Environ. Toxicol. Chem. 5:807-811.

Nebeker, A.V., S.T. Onjukka, M.A. Cairns and D.F. Krawczyk. 1986b. Survival of *Daphnia magna* and *Hyalella azteca* in cadmium-spiked water and sediment. Environ. Toxicol. Chem. 5:933-938.

Neville, C.M. 1985. Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum - prediction of field responses from laboratory data. Can J. Fish. Aquat. Sci. 42:2004-2019.

Niimi, A.J. and L. Lowe-Jinde. 1984. Differential blood cell ratios of rainbow trout (*Salmo gairdneri*) exposed to methylmercury and chlorobenzenes. Arch. Environ. Contam. Toxicol. 13:303-311.

Nobel, P. and Hartsock. 1984. Physiological responses of Opuntia ficus-india to growth temperature (nocturnal carbon dioxide uptake). Physiol. Plantarum 60:98-105.

Nobel, P. and S. Long. 1985. Canopy structure and light interception. pp. 41-49. In: Techniques in bioproductivity and photosynthesis. J Coombs et al. (eds.).

NRCC. 1985. The role of biochemical indicators in the assessment of ecosystem health - their development and validation. Publ. No. NRCC 24371, Ottawa. 119 pp.

Olsson, P.-E. and C. Haux. 1985. Rainbow trout metallothionein. Inorganica Chimica Acta. 107:67-71.

Olsson, P.-E. and C. Haux. 1986. Increased hepatic metallothionein content correlates to cadmium accumulation in environmentally exposed perch (*Perca fluviatilis*). Aquat. Toxicol. 9:231-242.

Olsson, P.-E., C. Haux and L. Forlin. 1986. Variations in hepatic metallothionen, zinc and copper levels during an annual reproductive cycle in rainbow trout, *Salmo gairdneri*. Fish. Physiol. Biochem. 1:00-00.

Omernik, J. 1987. Ecoregions of the coterminous United States. Ann. Assn. Amer. Geograph. 77:118-125.

Orciari, R.D. 1979. Rotenone resistance of golden shiners from a periodically reclaimed pond. Trans. Am. Fish. Soc. 108:641-645.

Orsatti S.D. and P.W. Colgan. 1987. Effects of sulphuric acid exposure on the behaviour of largemouth bass, *Micropterus salmoides*. Environ. Biol. Fish. 19:119-129.

Ottaway, E.M. and K. Simkiss. 1977a. "Instantaneous" growth rates of fish scales and their use in studies of fish populations. J. Zool. (London). 181:407-419.

Ottaway, E.M. and K. Simkiss. 1977b. A method for assessing factors influencing false "check" formation in fish scales. J. Fish Biol. 11:681-687.

Ottaway, E.M. and K. Simkiss. 1979. A comparison of traditional and novel ways of estimating growth rates from scales of natural populations of young bass (*Dicentranchus labiax*). J. Mar. Biol. Assoc. U.K. 59:49-59.

Overstreet, R.M. 1988. Aquatic pollution problems, Southeastern U.S. coasts: histopathological indicators. Aquat. Toxicol. 11:213-239.

Parker, D.B. and B.A. McKeown. 1987a. Effects of pH and/or calcium-enriched freshwater on plasma levels of vitellogenin and Ca^{2+} and on bone calcium content during exogenous vitellogenesis in rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 87A:267-273.

Parker, D.B. and B.A. McKeown. 1987b. The effects of low pH on egg and alevin survival of kokanee and sockeye salmon, *Oncorhynchus nerka*. Comp. Biochem. Physiol. 87C:259-268.

Parkhurst, B.R., H.L. Bergman, J. Fernandez, D.D. Gulley and J.R. Hockett. 1989. Evaluation of the *in situ* toxicity of acidic surface waters to brook trout. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 150.

Parsons, T.R., T.A. Kessler and L. Guanguo. 1986a. An ecosystem model analysis of the effect of mine tailings on the euphotic zone of a pelagic ecosystem. Acta Oceano. Sin. 5:425-436.

Parsons, T.R., P. Thompson, W. Yong, C.M. Lalli, H. Shumin and X. Huaishu. 1986b. The effect of mine tailings on the production of plankton. Acta Oceano. Sin. 5: 418-423.

Passino, D.R.M. 1984. Biochemical indicators of stress in fishes: an overview. pp. 37-50. In: V.W. Carrus, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries. Adv. Env. Sci. Tech. 16, Wiley & Sons, Toronto.

Pastor, A., J. Medina, J. Del Ramo, A. Torreblanca, J. Díaz-Mayans and F. Hernandez. 1988. Determination of lead in treated crayfish *Procambarus clarkii*: accumulation in different tissues. Bull. Environ. Contam. 41:412-418.

Payne, J.F. 1984. Mixed-function oxygenases in biological monitoring programs: review of potential usage in different phyla of aquatic animals. In: G. Persoone, E. Jaspers and C. Claus (eds.). Ecotoxicological Testing for the Marine Environment. State Univ. Ghent and Inst. Mar. Scient. Res., Bredene, Belgium. 1:789 pp.

Payne, J.F. and W.R. Penrose. 1975. Induction of aryl hydrocarbon (benzo(a)pyrene) hydroxylase in fish by petroleum. Bull. Environ. Contam. Toxicol. 14:112-116.

Payne, F. and Ford. 1988. The concept of TIME (Temporally Integrated Monitoring of Ecosystems). Supplement. Prepared for U.S. Environmental Protection Agency, Corvallis, OR. 70 pp.

Payne, J.F., L.L. Fancey, A.D. Rahimtula and E.L. Porter. 1986. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Unpubl. MS. pp. 2-47.

Pellerin-Massicotte, P., B. Vincent and M. Harvey. 1989. Ecotoxicological evaluation of a polluted ecosystem in the St. Lawrence estuary. 75 pp. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario.

Peterson, R.H., J.L. Metcalfe and S. Ray. 1983. Effects of cadmium on yolk utilization, growth, and survival of Atlantic salmon alevins and newly feeding fry. Arch. Environ. Contam. Toxicol. 12:37-44.

Pickering, A.D. (ed.). 1981. Stress and Fish. Academic Press, London, 367 pp.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid Bioassessment Protocols For Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. U.S. EPA, Washington, D.C., May, 1989. EPA/444/4-89-001.

Poulton, M.J. and D. Pascoe. 1989. Behaviour and early development in freshwater crustacea - modifying effects of cadmium. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 85.

Powell, J.F.F. and B.A. McKeown. 1986. The effects of acid exposure on the ion regulation and seawater adaptation of coho salmon (*Oncorhynchus kisutch*) parrs and smolts. Comp. Biochem. Physiol. 83C:45-52.

Powles, S. and O. Bjorkman. 1982. High light and water stress effects on photosynthesis. pp. 747-77. In: Nerium oliander. Carnegie Institute of Washington Year Book.

Price-Haughey, J., K. Bonham and L. Gedamu. 1986. Heavy metal-induced gene expression in fish and fish cell lines. Environ. Health Perspect. 65:141-147.

Pyron, M. and T.L. Beitinger. 1989. Effect of selenium on reproductive behaviour and fry of fathead minnows. Bull. Environ. Contam. Toxicol. 42:609-613.

Quillardet, P., O. Huisman, R. D'Ari and M. Hofnung. 1982. SOS chromotest, a direct assay of induction of an SOS function in *Eschericia coli* K-12 to measure genotoxicity. Proc. Nat. Acad. Sci. USA. 79:5971-5975.

Rabeni, C.F., S.P. Davies, K.E. Gibbs. 1985. Benthic invertebrate response to pollution abatement: Structural changes and functional implications. Water Resources Bulletin, American Water Resources Association. 21:489-497.

Raddum, G.G. and A. Fjellheim. 1987. Effects of pH and aluminium on mortality, drift and moulting of the mayfly *Baetis rhodani*. Annls. Soc. R. Zool. Belg. 117 (Suppl. 1): 77-88.

Rainbow, P.S. 1989. Biomonitoring and heavy metal accumulation strategies in crustaceans. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 64.

Ram, R.N and A.G. Sathyanesan. 1985. Mercurial induced brain monoamine oxidase inhibition in the teleost *Channa punctatus* (bloch). Bull. Environ. Contam. Toxicol. 35:620-626.

Ram, R.N. and K.P. Joy. 1988. Mercurial induced changes in the hypothalamo-neurohypophysical complex in relation to reproduction in the teleostean fish, *Channa punctatus* (bloch). Bull Environ. Contam. Toxicol. 41:329-336.

Rapport, D.J. 1989. Symptons of pathology in the Gulf of Bothnia (Baltic Sea): ecosystem response to stress from human activity. Biol. J. Linn. Soc. 37:33-49.

Reddy, R.S., R.R. Jinna, J.E. Uzodinma and D. Desaiah. 1988. *In vitro* effect of mercury and cadmium on brain Ca^{2+} ATPase of the catfish *Ictalurus punctatus*. Bull. Environ. Contam. Toxicol. 41:324-328.

Rehnberg, B.C. and C.B. Schreck. 1986. Acute metal toxicology of olfaction in Coho salmon: behavior, receptors, and odor-metal complexation. Bull. Environ. Contam. Toxicol. 36:579-586.

Reid, S.D. and D.G. MacDonald. 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can J. Fish. Aquat. Sci. 45:244-253.

Rhee, G.Y., M. Munawar and P.T.S. Wong 1989. Physiological responses of phytoplankton to contaminants. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 162.

Ridlington, J.W., D.E. Chapman, B.L. Boese, V.G. Johnson and D.R. Randall. 1982. Petroleum refinery wastewater induction of the hepatic mixed-function oxidase system in Pacific staghorn sculpin. Arch. Environ. Contam. Toxicol. 11:123-127.

Roch, M. and J.A. McCarter. 1984a. Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) 1. exposed to an artificial mixture of zinc, copper and cadmium. Comp. Biochem. Physiol. 77C:71-75.

Roch, M. and J.A. McCarter. 1984b. Hepatic metallothionein production and resistance to heavy metals by rainbow trout (*Salmo gairdneri*) 2. held in a series of contaminated lakes. Comp. Biochem. Physiol. 77C:77-82.

Roch, M. and J.A. McCarter. 1984c. Metallothionein induction, growth and survival of chinook salmon exposed to zinc, copper and cadmium. Bull. Environ. Contam. Toxicol. 32:478-485.

Roch, M., P. Noonan and J.A. McCarter. 1986. Determination of no effect levels of heavy metals for rainbow trout using hepatic metallothionein. Wat. Res. 20:771-774.

Roch, J., J.A. McCarter, A.T. Matheson, M.J.R. Clark and R.W. Olafson. 1982. Hepatic metallothionein in rainbow trout (*Salmo gairdneri*) as an indicator of metal pollution in the Campbell River system. Can J. Fish. Aquat. Sci. 39:1596-1601.

Rõderer, G. 1981. On the toxic effects of tetraethyl lead and its derivatives on the chrysophyte *Poterioochromonas malhamensis*. II. Triethy lead, diethy lead and inorganic lead. Environ. Res. 25:361-371.

Rõderer, G. 1986. On the toxic effects of tetraethyl lead and its derivatives on the chryosophyte *Poterioochromonas malhamensis*. Environ. Res. 39:205-231.

Sanders, B.M. 1989. Stress proteins in biomonitoring. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 174.

Saunders, R.L. and J.B. Sprague. 1967. Effects of copper-zinc mining pollution on a spawning migration of Atlantic salmon. Water Res. 1:419-432.

Schindler, D.W. 1987. Detecting ecosystem responses to anthropogenic stress. Can. J. Fish. Aquat. Sci. 44(Suppl. 1):6-25.

Schmitt, C.J., F.J. Dwyer and S.E. Finger. 1984. Bioavailability of Pb and Zn from Mine Tailings as indicated by erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). Can J. Fish. Aquat. Sci. 41:1030-1040.

Schom, C.B. 1986. Genetic, environmental, and maturational effects on Atlantic salmon (*Salmo salar*) survival in acute low pH trials. Can. J. Fish. Aquat. Sci. 43:1547-1555.

Scudder, B.C., J.L. Carter and H.V. Leland. 1988. Effects of copper on development of the fat head minnow, Pimphales promelas Rafinesque. Aquat. Toxicol. 12: 107-124.

Seim, W.K., L.R. Curtis, S.W. Glenn and G.A. Chapman. 1984. Growth and survival of developing steelhead trout (*Salmo gairdneri*) continuously or intermittently exposed to copper. Can. J. Fish. Aquat. Sci. 41:433-438.

Servos, M.R. and G.L. Mackie. 1986. The effect of short-term acidification during spring snowmelt on selected Mollusca in south-central Ontario. Can J. Zool. 64:1690-1695.

Shephard, K.L. 1987. The influence of water pH on the perivitelline fluid of perch (*Perca fluviatilis*) eggs. Comp. Biochem. Physiol. 86C:383-386.

Sheridan, R.P. 1978. Toxicity of bisulfite to photosynthesis and respiration. J. Phycol. 14:279-281.

Sicko-Goad, L., B.G. Ladewski and D. Lazinsky. 1986. Synergistic effects of nutrients and lead on the quantitative ultrastructure of *Cyclotella* (Bacillariophyceae). Arch. Environ. Contam. Toxicol. 15:291-300.

Siddens, L.K., W.K. Seim, L.R. Curtis and G.A. Chapman. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 43:2036-2040.

Silver, M. 1989. Control of acid mine drainage including coal pile and ash pond seepage: biology and chemistry of generation, prevention and abatement of acid mine drainage. pp 753-760. In: Hammer, D.A. (ed.), Constructed Wetlands for Wastewater Treatment: municipal, industrial, and agricultural. Proc. First Internat. Conf. Constructed Wetlands for Wastewater treatment, Chattanooga, TN, June 13-17, 1988.

Sindermann, C.J. 1988. Biological indicators and biological effects of estuarine/coastal pollution.l Wat. Res. 24:931-939.

Sinley, J.R., J.P. Goettl and P.H. Davies. 1974. The effects of zinc on rainbow trout (*Salmo gairdneri*) in hard and soft water. Bull. Environ. Contam. Toxicol. 12:192-201.

Skurdal, J., O.K. Skogheim and T. Qvenild. 1986. Indicative value of mercury concentration in adipose fin of brown trout, *Salmo trutta* L. J. Fish. Biol. 29:515-517.

Slabbert, J.L. and W.S.G. Morgan. 1982. A bioassay technique using *Tetrahymena pyriformis* for the rapid assessment of toxicants in water. Water Res. 16:517-523.

Slabbert, J.L. and W.O.K. Grabow. 1986. A rapid water toxicity screening test based on oxygen uptake of *Pseudomonas putida*. Toxicity Assess. 1:13-26.

Slater, T.E. 1978. Biochemical studies on liver injury. pp. 2-44. In: T.F. Slater (ed.). Biochemical Mechanisms of Liver Injury. Academic Press, New York.

Sloley, B.D., B.E. Hickie, D.G. Dixon, R.G.H. Downer and R.J. Martin. 1986a The effects of sodium pentachlorophenate, diet and sampling procedure on amine and tryptophan concentrations in the brain of rainbow trout, *Salmo gairdneri* Richardson. J. Fish. Biol. 28:267-277.

Sloley, B.D., R.A. Cunjak, G. Power and R.G.H. Downer. 1986b. The influence of sex and spawning on levels of tryptophan, serotonin and 5-hydroxyindoleacetic acid in the brains of wild brook trout, *Salvelinus fontinalis*. J. Fish Biol. 29:663-669.

Slooff, W. 1982. Skeletal anomalies in fish from polluted surface waters. Aquat. Toxicol. 2:157-173.

Smith, R.J.F. and B.J. Lawrence. 1988. Effects of acute exposure to acidified water on the behaviourial response of fathead minnows, *Pimephales promelas*, to alarm substance (Schreckstoff). Environ. Toxicol. Chem. 7:329-335.

Sonstegard, R.A. and J.F. Leatherland. 1984. Comparative epidemiology: the use of fishes in assessing carcinogenic contaminants. p. 223-231. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.). Contaminant Effects on Fisheries. Adv. Environ. Sci. Technol. Vol. 16.

Sorensen, E.M.B. and T.L. Bauer. 1983. Hematological dyscrasia in teleosts chronically exposed to selenium-laden effluent. Arch. Environ. Contam. Toxicol. 12:135-141.

Spear, P.A. and R.C. Pierce. 1979. Copper in the aquatic environment: chemistry, distribution and technology. NRCC No. 16454. National Research Council of Canada, Ottawa.

Spehar, R.L. and J.T. Fiandt. 1986. Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Environ. Toxicol. Chem. 5:917-931.

Spies, R.B., J.S. Felton and L. Dillard. 1982. Hepatic mixed function oxidases in California flatfishes are increased in contaminated environments and by oil and PCB ingestion. Mar. Biol. 70:117-127.

Sprague, J.B. 1976. Current status of sublethal effects of pollutants on aquatic organisms. J. Fish. Res. Board Can. 33:1988-1992.

Sprague, J.B. 1985. Chapter 6: Factors that Modify Toxicity. pp. 124-157. In: G.M. Rand and S.R. Petrocelli (eds.), Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Publishing Corporation, New York, N.Y.

Sprague, J.B., P.F. Elson and R.L. Saunders. 1965. Sublethal copper-zinc pollution in a salmon river - A field and laboratory study. Air Water Pollut. 9:531-543.

Spry, D.J. and K.M. Miller. 1989. Acute toxicity of acid to early life stages of several cyprinid species in natural soft water. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 243.

Stagg, R.M. and T.J. Shuttleworth. 1982. The accumulation of copper in *Platichthys flesus* L. and its effects on plasma electrolyte concentrations. J. Fish. Biol. 20:491-500.

Stebbing, A.R.D. 1982. Hormesis - the stimulation of growth by low levels of inhibitors. Sci. Total Environ. 22:213-234.

Steele, C.W., D.W. Owens, A.D. Scarfe and P. Thomas. 1985. Behavioural assessment of the sublethal effects of aquatic pollutants. Mar. Poll. Bull. 16:221-224.

Stegeman, J.J. 1978. Influence of environmental contamination on cytochrome P-450 mixed function oxygenases in fish: implications for recovery in the Wild Harbour Marsh. J. Fish. Res. Board Can. 35:668-674.

Tam, W.H., L. Birkett, R. Makaran, P.D. Payson, D.K. Whitney and C.K.-C Yu. 1987. Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (*Salvelinus fontinalis*) by exposure to low pH. Can. J. Fish. Aquat. Sci. 44:630-635.

Taub, F. 1989. Standardized aquatic microcosm - development and testing. In: Aquatic Ecotoxicology: Fundamental Concepts and Methodologies, Volume II. A Boudou and F Ribeyre (eds.), CRC Press. In press.

Taub, F.A., F. Kindig, F. Conquest and J. Meador. 1989. Results of interlaboratory testing of the standardized aquatic microcosm protocol. pp. 368-394. In: Aquatic Toxicology and Environmental Fate: Eleventh Volume, ASTM, STP 1007. GW Suter II and MA Lewis (eds.), American Society for Testing and Materials, Philadelphia.

Taylor, D.H., C.W. Steele and S. Strickler-Shaw. 1989. Attraction of crayfishes to a feeding stimulant, and its suppression by a blend of metals. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 84.

Tessier, A. and P.G.C. Campbell. 1989. Environmental and biological factors affecting the bioaccumulation of metals by freshwater molluscs. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 154.

Thornton, K., F. Payne, J. Ford and D. Landers. 1987. The Concept of Time: Temporally Integrated Monitoring of Ecosystems. Corvallis Environmental Research Laboratory, Corvallis Oregon.

Torreblanca, A., J. Del Ramo and J. Díaz-Mayans. 1989. Gill ATPase activity in *Procambarus clarkii* as an indicator of heavy metal pollution. Bull. Environ. Contam. Toxicol. 42:829-834.

Towle, D.W. 1981. Role of Na+, K+-ATPase in ionic regulation by marine and estuarine animals. Mar. Biol. Lett. 2:107-122.

Trippel, E.A. and H.H. Harvey. 1987a. Reproductive responses of five white sucker (*Catostomus commersoni*) populations in relation to lake acidity. Can. J. Fish. Aquat. Sci. 44:1018-1023.

Trippel, E.A. and H.H. Harvey. 1987b. Abundance, growth, and food supply of white suckers (*Catostomus commersoni*) in relation to lake morphometry and pH. Can J. Zool. 65:558-564.

Turbak, S.C., S.B. Olson and G.A. McFeters. 1986. Comparison of algal assay systems for detecting waterborne herbicides and metals. Wat. Res. 20:91-96.

Unni, L.K. and M.L Caspers. 1985. Inhibition of bovine monoamine oxidase levels by lead. Biochem. Pharmacol. 34:2563-2566.

Verma, S.R., R. Chand and I.P. Tonk. 1986. Mercuric chloride-induced physiological dysfunction in *Notopterus notopterus*. Environ. Res. 36:442-447.

Versteeg, D.J. and J.P. Giesy. 1986. The histological and biochemical effects of cadmium exposure in the bluegill sunfish (*Lepomis macrochirus*). Ecotoxicol. Environ. Saf. 11:31-43.

Vetter, R.D. and R.E. Hodson. 1984. Metabolic indicators of sublethal stress: changes in adenine nucleotodes, glycogen and lipid. pp. 471-498. In: H.H. White (ed.). Concepts in marine pollution measurements. Maryland Sea Grant College, University of Maryland, College Park, Maryland.

Vigers, G.A., J. Barrett, R. Hoffman, J. Humphrey, D. Kathman, D. Konasewich, R. Olmstead and B. Reid. 1983. Toxicological, biophysical and chemical assessment of Red Dog Creek, DeLong Mountains, Alaska, 1982. Prepared for Alaska Department of Environmental Conservation, Juneau. 245 pp.

Voss, M., G. Renger, C. Kotter and P. Graber. 1984. Fluorometric detection of photosystem II herbicide penetration and detoxification in whole leaves. Weed Sci. 32:675-680.

Walker, J.D. 1988. Relative sensitivity of algae, bacteria invertebrates, and fish to phenol: analysis of 234 tests conducted for more than 149 species. Toxicity Assess. 3:415-447.

Walsh, G. 1983. Cell death and inhibition of population growth of marine unicellular algae by pesticides. Aquat. Toxicol. 3:290-214.

Walsh, G. 1988a. Methods for toxicity tests of single substances and liquid complex wastes with marine unicellular algae. EPA/600/8-87/043.

Walsh, G. 1988b. Principles of toxicity testing with marine unicellular algae. Environ. Toxicol. Chem. 7:979-987.

Walsh, G., D. Hansen and D. Lawrence. 1982. A flow-through system for exposure of seagrass to pollutants. Mar. Env. Res. 7:1-11.

Walsh, J., C. Wirick, L. Pietrafesa, T. Whitledge, F. Hoge and R. Swift. 1988. High-frequency sampling of the 1984 spring bloom within the Mid-Atlantic Bight: a synoptic shipboard, aircraft, and *in situ* perspectives of the SEEP-I experiment. Cont. Shelf Res. 8:529-563.

Warren, C.E. and G.E. Davis. 1967. Laboratory studies on the feeding bioenergetics and growth of fish. pp. 175-214. In: S.D. Gerking (ed.). The Biological Basis of Freshwater Fish Production. Blackwell Scientific, Oxford.

Warren-Hicks, W.J., R.L. Wolpert, J.P. Baker and K.H. Reckhow. 1989. Estimating fish response to acidification: using bayesian inference to develop models incorporating both laboratory bioassay and field measurements of fish response. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario. p. 106.

Watras, C.J., J. MacFarlane and F.M.M. Morel. 1985. Nickel accumulation by *Scenedesmus* and *Daphnia*: food-chain transport and geochemical implications. Can. J. Fish. Aquat. Sci. 42:724-730.

Wedemeyer, G.A., D.J. McLeay and C.P. Goodyear. 1984. Assessing the tolerance of fish and fish populations to environmental stress: the problems and methods of monitoring. pp. 163-195. In: V.W. Cairns, P.V. Hodson and J.O. Nriagu (eds.), Contaminant Effects on Fisheries, Advances Environ. Sci. Technol. Vol. 16.

Weis, P., J.D. Bogden and E.C. Enslee. 1986. Hg- and Cu-induced hepatocellular changes in the mummichog, *Fundulus heteroclitus*. Environ. Health. Perspect. 65:167-173.

Weis, J.S. and P. Weis. 1987. Pollutants as developmental toxicants in aquatic organisms. Environ. Health Perspect. 71:77-85.

Weis, J.S. and P. Weis. 1989. Tolerance and stress in a polluted environment. Bioscience. 39:89-95.

Weiser, W. and S. Hinterleitner. 1980. Serum enzymes in rainbow trout as tools in the diagnosis of water quality. Bull. Environ. Contam. Toxicol. 25:188-193.

Whitledge, T. and C. Wirick. 1986. Development of a moored in situ fluorometer for phytoplankton studies. pp. 449-462. In: J. Bowman, M Yentsch and W Peterson (eds.), Lecture Notes on Coastal and Estuarine Studies, Vol. 17: Tidal Mixing and Plankton Dynamics. Sringer-Verlag, Berlin Heidelberg.

Whittier, T., R. Hughes and D. Larsen. 1988. Correspondence between ecoregions and spatial patterns in stream ecosystems in Oregon. Can. J. Fish. Aquat. Sci. 45:1264-1278.

Widdows, J. 1985a. Physiological responses to pollution. Mar. Pollut. Bull. 16:129-134.

Widdows, J. 1985b. Physiological measurements and physiological procedures. pp. 3-45 and 161-178. In: B.L. Bayne et al. (eds.). The Effects of Stress and Pollution on Marine Animals. Praeger Press, New York.

Widdows, J., D.K. Phelps and W. Galloway. 1981. Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. Mar. Environ. Res. 4:181-194.

Widdows, J., P. Donkin, P.N. Salkeld, J.J. Cleary, D.M. Lowe, S.V. Evans and P.E. Thomson. 1984. Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). Mar. Ecol. Prog. Ser. 17:33-47.

Wilhm, J.L. 1975. Biological indicators of pollution. pp. 375-402. In: B.A. Whitton (ed.) River Ecology. University of California Press. Berkley.

Wilson, D. 1980. Copper, zinc and cadmium concentrations of resident trout related to acid-mine wastes. Calif. Fish Game J. 67:176-86.

Winner, R.W. 1984. The toxicity and bioaccumulation of cadmium and copper as affected by humic acid. Aquat. Toxicol. 5:267.

Wolf, P.L., D. Williams and E. Von der Muehll. 1973. Practical clinical enzymology. John Wiley and Sons, Toronto, Ontario. 580 pp.

Woock, S.E., W.R. Garrett, W.E. Partin and W.T. Bryson. 1987. Decreased survival and teratogenesis during laboratory selenium exposures to bluegill, *Lepomis macrochirus*. Bull. Environ. Contam. Toxicol. 39:998-1005.

Wood, C.M. and M.S. Rogano. 1986. Physiological responses to acid stress in crayfish (*Orconectes*): haemolymph ions, acid-base status, and exchanges with the environment. Can. J. Fish. Aquat. Sci. 43:1017-1026.

Wren, C.D. and H.R. MacCrimmon. 1986. Comparative bioaccumulation of mercury in two adjacent freshwater ecosystems. Water Res. 20:763-769.

Xu, H., B.J. Dutka and K.K. Kwan. 1987. Genotoxicity studies on sediments using a modified SOS chromotest. Toxicity Assess. 2:79-87.

Yount, J. and J. Richter. 1986. Effects of pentachlorophenol on periphyton communities in outdoor experimental streams. Arch. Environ. Contam. Toxicol. 15:51-60.

Youson, J.H. and C.M. Neville. 1987. Deposition of aluminum in the gill epithelium of rainbow trout (*Salmo gairdneri* Richardson) subjected to sublethal concentrations of the metal. Can J. Zool. 65:647-656.

Zinkl, J.G. 1989. The use of cholinesterase to evaluate poisoning of fish by organophosphate and carbamate insecticides. 149 pp. In: Transboundary Pollution, Abstracts of 10th Annual Meeting. Soc. Environ. Toxicol. Chem., Oct. 28-Nov. 2, 1989, Toronto, Ontario.

8.0 GLOSSARY

a priori	Deductive; derived in advance by reasoning.
acetylcholinesterase	An enzyme which breaks down the neurotransmitter acetylcholine.
achromia	Lacking colour or usual pigmentation.
adenylate energy charge	The ratio of adenosine triphosphate (ATP) to the total adenylates (AMP + ADP + ATP).
ALAD	δ-aminolevulinic acid dehydratase; an enzyme which condenses 2 molecules of aminolevulinic acid to form one molecule of porphobilinogen, a precursor of haem rings used in haemoglobin synthesis.
allantoinase	An enzyme involved in purine and nitrogen metabolism.
algae	Aquatic, nonflowering plants which lack roots and live on inorganic nutrients such as nitrogen and phosphorous and produce organic matter by photosynthesis. Common algae include those that are single celled (e.g. dinoflagellates, diatoms) and larger algae (e.g. seaweeds and macrophytes). An algal bloom can occur when excessive nutrients and certain water conditions enable the organisms to reproduce rapidly.
alkaline phosphatase	Enzymes which break down adenosine triphosphate for energy
ALT	Aspartate aminotransaminase; a cellular enzyme normally found in low levels in the blood. Elevated levels suggest tissue damage.
ambient	Surrounding; on all sides.
Ames test	A bacterial assay for mutagenicity using strains of *Salmonella typhimurum.*
anaemia	A significant decrease in the number of erythrocytes per volume of blood.
anisocytosis	A condition where adjacent cells are unlike.
AST	Alanine transaminase; a cellular enzyme normally found in low levels in the blood. Elevated levels suggest tissue damage.
ataxia	Total or partial inability to coordinate voluntary bodily movements.
axial muscle	The muscles in vertebrates which move the axial skeleton (skull, vertebral column, sternum and ribs). In fish these muscles are responsible for propulsion.
Benthic	Referring to organisms living in or on the sediments of aquatic/marine habitats.
BGSD	ß-galactosidase; an enzyme used in the SOS-chromotest system.
bioassay	Any test which uses organisms (plants, animals, tissues) to measure the toxicity of a material. A bioassay test is used to measure a degree of response produced by exposure to a sample, a specific level of stimulus or a chemical concentration.

bioconcentration	The accumulation in an aquatic organism of a chemical taken up directly from the water.
biomagnification	The accumulation in an aquatic organism of a chemical taken up through diet (via the food chain).
branchiostegal ray	Bony structures along the internal, ventral surface of fish operculae.
carbamate pesticides	A group of pesticides which act as inhibitors of acetylcholinesterase by reversible binding to active sites of the enzyme.
carcinoma	Any of several kinds of cancerous growths made up of epithelial cells (cellular tissue covering surfaces, forming glands, and lining most cavities in the body).
catabolism	The process in a plant or animal by which living tissue is broken down into various products. Sometimes the release of energy or the metabolites produced in this process are used in the cell and sometimes the products are excreted.
catecholamines	A group of diphenylalkyl amine hormones (including epinephrine and norepinephrine) which are used as neurotransmitters in fish neural tissue.
chelate	The type of coordination compound in which a central metal ion is attached by coordinate links to two or more non-metal atoms in the same molecule, called ligands.
Chironomidae	A family of freshwater insects which spend most of their life-cycle in rivers, lakes and ponds. Also known as midges.
chlorosis	An abnormal condition of plants in which the green parts lose their colour or turn yellow as a result of a disease or lack of light.
circadian	Daily cycle.
coenzyme	An organic substance of low molecular weight that is not protein and that can unite with a given protein to form an active enzyme system
congenital	Present at birth.
corticosteroid	Any of the hormones secreted by the adrenal cortex; in fish these are produced by interrenal tissue.
cuticular proteins	Any of the proteins that make up the tough, nonliving outer structure (cuticle) secreted by the epidermis in many invertebrate organisms.
cytological	Concerning cells.
daphnia	A freshwater micro-crustacean found in ponds, lakes or streams. Sometimes referred to as water fleas, includes *Daphnia* and *Ceriodaphnia*, two common bioassay organisms.
depuration	Elimination of material from the digestive tract of an aquatic organism.
diatom	Any of a number of related microscopic algae whose cell walls consist of two boxlike parts or valves and contain silica.

electroshocking	A technique used to sample fish. A current is passed through the water and the stunned fish usually rise to the top of the water where they are collected.
epizootiology	The study of epidemic animal diseases.
Ephemeroptera	An order of insects which spends most of its life-cycle in rivers, lakes and streams. This order includes the mayflies.
erythrocyte	Red blood cell.
erythropoiesis	The body process of developing red blood cells.
estradiol	A female sex hormone.
estuarine	Residing or situated in a semi-enclosed coastal body of water which has a free connection with the open sea and within which seawater is measurably diluted with freshwater derived from land drainage.
eutrophication	The process of nutrient enrichment that causes high productivity and biomass in an aquatic ecosystem. Eutrophication can be a natural process or it can be a cultural process accelerated by an increase in nutrient loading to a water body by human activity.
fasciculations	Small bundles.
fecundity	Total number of eggs produced by a female.
gonadosomatic index	GSI; a ratio of gonad weight to total body weight.
GOT	Glutamic oxaloacetic transaminase; a cellular enzyme normally found in low levels in the blood. Elevated levels suggest tissue damage.
GPT	Glutamic pyruvic transaminase; a cellular enzyme normally found in high levels in the liver and in low levels in the blood. Elevated levels suggest tissue damage.
haematocrit	Packed volume, following centrifugation, of circulating blood cells; expressed as a percentage of the whole blood volume. Values correspond primarily to erythrocyte (red blood cell) content.
haematology	The study of blood.
haemoglobin	A protein found in red blood cells which binds either molecular oxygen or carbon dioxide and releases carbon dioxide to the atmosphere and oxygen to tissues.
haemolymph	Denoting the flowing tissue in both the circulatory system and the lymphatic system. Blood and lymph.
histological	Concerned with the microscopic study of the structure of tissues.
in situ	"In place." Usually used to distinguish work conducted "in the field" from work done in the laboratory.

in vitro	Outside the intact organism; generally applied to experiments involving biochemical events occurring in tissue fragments or fractions.
in vivo	Within an intact animal or organism.
indoleamines	A group of hormones derived from tyrosine (including serotonin and 5-hydroxyindole acetic acid) which are used in the formation of neurotransmitters, or as neurotransmitters, in fish and invertebrates.
INT	A dye used for assessing the activity of dehydrogenase enzymes; reduction of the parent compound results in a change in the colour of the dye, which is determined spectrophotometrically.
intraperitoneal	Inside the body cavity.
ionoregulatory	Concerning the maintenance of certain concentrations of ions in various tissues throughout the body.
isoforms	Several forms of the same thing.
jaw gape	Opening for the mouth.
kyphosis	Backward curvature of the spine; humpback.
LAN	Leucine (L-) aminonapthylamidase; an enzyme usually found in lysozymes. Increased blood levels are a sign of tissue damage.
leachates	A soluble material, such as organic and mineral salts, which is washed out of a layer of soil or debris.
leucocrit	Packed volume, following centrifugation, of circulating white blood cells (leucocytes), expressed as a percentage of the whole blood volume.
ligands	A molecule, ion or atom that is attached to the control atom of a coordination compound, a chelate or other complex. May also be called a chelating agent.
lipophilic	Compounds or molecules which preferentially associate with lipids (fats).
liversomatic index	LSI; a ratio of liver weight to total body weight.
lordosis	An anteroposterior curvature of the spine, generally in the lumbar region. Also called hollow back or saddle back.
lysosomes	Structures found in the cytoplasm of cells and containing a number of digestive enzymes capable of breaking down most of the constituents of living matter.
macroinvertebrates	Invertebrate (without backbone) animals that are not microscopic, includes insects and oligochaetes, among others.
macrophages	Any of a number of cells in connective tissue, bone marrow, lymphatic tissue etc. which ingest and destroy other cells, microorganisms and or other foreign matter in the blood and other tissues.
macrozoobenthos	Large benthic invertebrates like Plecopterans and Ephemeropterans.

malathion	A widely used organophosphate pesticide.
mantle	The glandular flap or folds of the body wall of a mollusc which typically secrets a shell-forming fluid.
mean corpuscular volume	A measure of the size of blood cells.
meristic characters	Any of a number of characteristics of fish which can be counted; number of scales in the lateral line, numbers of gill filaments, etc.
mesocosm	A simulated micro-environment used to produce an artificially ecosystem for toxicity testing.
metallothionein	An inducible protein, rich in cysteine residues (high in sulphur content), which is produced by various tissues in response to elevated levels of certain metals (Cd, Cu, Ag and Zn).
methoxychlor	An organochloride pesticide (derivative of DDT) which has been widely used to control blackflies.
microcosm	A small ecosystem, developed for field-scale manipulations in a controlled system.
mitotic	Mitosis; process of cell division which produces two identical, diploid cells from one original.
MSH	Melanocyte stimulating hormone; a hormone produced by the pituitary to regulate a number of physiological processes, including the formation and pigmentation of melanocytes.
mutagen	A chemical that causes an alteration of the inherited genetic material, i.e., the DNA of the genes. In the narrow sense, the chemical alters the genetic material of paternal or maternal sex cells.
myelin	The white fatty substance which forms a sheath around certain nerve fibres.
necrosis	The death or decay of tissue in a particular part of the body.
neoplasm	A tumorous cell growth.
neoplastic	Pertaining to or like a neoplasm.
neurotoxic	Harmful to the workings of the nervous system in some way.
Odonata	An order of freshwater insects which spend a portion of their lives on the bottom of rivers, ponds and lakes. This order includes dragonflies and damselflies.
oedema	An abnormal accumulation of fluid in cells, tissues or cavities of the body, resulting in swelling.
olfaction	Smelling, the sense of smell.
oligotrophic	Waters with a low supply of nutrients.

opercular rhythms	Frequency and intensity of breathing movements in fish.
optical attenuation	Diminishing of light.
organophosphate pesticides	A group of pesticides which act as irreversible inhibitors of acetylcholinesterase by binding to the active site of the enzyme.
osmolality	Osmolarity; the concentration of dissolved solids in a solution.
osmoregulatory	Pertaining to or involved in the regulation of osmotic pressure within an organism, accomplished by proper water and electrolytic balance.
parathion	A widely-used organophosphate pesticide.
periphyton	Attached freshwater or marine algae; mainly filamentous.
pH	A measure of the strength of acidity or alkalinity of a solution; the $-\log_{10}$ concentration of the H^+ ion concentration.
polichromatophilia	Several histological staining characteristics.
prolactin	A pituitary hormone stimulating milk secretion in mammals and secretion by the crop gland in certain birds; used for ionic control in fish.
proteolytic enzyme	An enzyme which breaks down proteins into simpler substances.
protoporphyrin	Intermediate compound in the formation of porphyrin rings for the formation of the haem components of haemoglobin.
protozoan	Any member of the phylum Protozoa comprising all unicellular or acellular animals. It includes rhizopods, flagellates, sporozoans and ciliates.
radioimmunoassay	RIA; a method of measuring minute concentrations of biochemicals involving the use of radioactive (commonly H^3) antibodies.
scoliosis	Lateral curvature of the spine.
scrapers	Invertebrates that feed on periphyton and bacteria attached to plants and rocks.
secondary lamellae	Secondary gill filaments.
serum	The fraction of blood or lymph which does not consist of cells or fibrinogen.
shredders	Invertebrates that feed on plants by tearing off small pieces with their sharp mouth parts before ingestion.
sorbitol dehydrogenase	Liver enzyme used for interconversion of fructose and sucrose.
stoichiometric	The determination of the proportions in which chemical elements combine and the weight relations in any chemical reaction.

suspended solids	Organic or inorganic particles that are suspended in and carried by the water. The term includes sand, silt, and clay particles as well as solids in wastewater.
teratogen	An agent which increases the incidence of congenital malformations.
thigmotaxis	Stereotaxis; orientation and movement of an organism with respect to a solid object.
thylakoid	Vesicle or wall of photosynthetic pigments.
transaminase enzymes	Enzymes used in the interconversion of amino acids.
TTC	A dye used for assessing the activity of dehydrogenase enzymes; reduction of the parent compound results in a change in the colour of the dye, which is determined spectrophotometrically.
tubificid	An aquatic oligochaete or sludge worm which is tolerant to organically enriched waters.
vasculature cannulation	Insertion of a tube into a blood vessel for the purposes of repetitive administration of compounds or serial sampling of blood.
visceral	Pertaining to the body cavity.
vitellogenin	A large lipophosphoprotein yolk precursor produced in the liver of female fish.
xenobiotic	A foreign chemical or material not produced in nature and not normally considered a constitutive component of a specified biological system. This term is usually applied to manufactured chemicals.